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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Contract No. NAS 9 8200:ML

August 1, 1969 through August 30, 1970

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by

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FOREWORD

This is the second annual report on the project NAS 9 8200 entitled "Study to Define and Verify the Personal Oral Hygiene Requirements for Extended Manned Space Flight."

The investigators wish to acknowledge the contributions of Mr. John Allen in the design and fabrication of modifications of the hypobaric pressure chamber. The authors also wish to thank Dr. John Simpson for carrying out carbohydrate determinations on space diets and Dr. Samuel Dreizen, Mrs. Mary Fuller, and Miss Barbara Hellmers for their assistance in the preparation of this report.

STUDY TO DEFINE AND VERIFY THE PERSONAL ORAL HYGIENE REQUIREMENTS FOR EXTENDED MANNED SPACE FLIGHT

INTRODUCTION

Extended space travel is verging on reality. It has become important, therefore, to establish whether space flight conditions will create health hazards originating from the oral cavity. As part of a program intended to define the oral hygiene requirements for extended manned spacecraft flights, laboratory studies are being conducted in small primates housed in a hypobaric chamber constructed to simulate the spacecraft environment. This model system has been used in four experiments with marmosets. The primary objectives were to obtain a marmoset oral microbial census before, during, and after isolation in the space-simulated environment and to test sampling technics which would be applicable to man in land-based spacecraft simulators or in actual space flight. The same microbiologic sampling procedures used for the marmosets are being evaluated in humans under conventional and unconventional environmental conditions. The subjects include normal (healthy) individuals, cancer patients confined to hospital quarters, and cancer patients isolated in protected environments (sterile life island units or sterile laminar air flow rooms).

Studies are being designed and initiated to assess practical and adequate means of preventing potential intraoral health hazards during space flight by appropriate oral hygiene methods. The effectiveness of physico-chemical methods for controlling the oral microflora and the influence of currently used space diets on dental plaque accumulation and oral health are under active investigation.

The first annual NASA Report[1] contained preliminary data relative to the sampling technics, microbiologic procedures, fabrication and operation of the hypobaric chamber, and the results of one two-week chamber trial. The current report contains (1) additional data regarding the sampling procedures and comparison of the oral microflora of man and marmoset, (2) the cumulative findings from three two-week and one four-week chamber tests with marmosets, (3) comparison of representative oral microflora in normal subjects and in cancer patients exposed to conventional and protected environments, and (4) preliminary results of the retention of microorganisms on toothbrushes and the plaque forming potential of space diets.

PROCEDURES

1. Microbial Sampling, Transporting and Culturing Procedures for Comparing the Oral Flora of Man and Marmoset.

To obtain oral specimens for microbiologic analyses from both marmosets and humans, it was necessary to develop comparable, uncomplicated sampling methods. Logistical problems stemming from the eventual need to procure and transport specimens from subjects isolated in hermetically sealed, land-based spacecraft simulators to distant laboratory testing sites, necessitated the screening of transport or maintenance media which would least alter the specimens prior to analysis. Various methods were evaluated from the standpoint of quantitative and qualitative microbial reproducibility.

The sampling procedures ultimately selected for use in man and marmoset were: (a) 2 mm wire loops for saliva; (b) 3 mm paper points for gingival sulcus fluid; and (c) calcium alginate swabs (Calgiswab #11-60E)[a/] for intraoral surfaces.

The oral specimens were suspended in 2 ml of 0.1 per cent peptone in normal saline and kept in cracked ice during transport or maintenance periods of less than one hour. The suspensions then were appropriately diluted in the same medium and plated on solid media using glass rod spreaders for dispersion. Details of these procedures were presented in the first annual report to NASA.[1]

Stimulated human saliva obtained by chewing rubber bands served as a reference or baseline specimen. Recently oral mucus was compared to stimulated saliva as a method of assessing the oral microflora. Mucus was collected by 30 second sequential saline mouth rinses over a five-minute period according to the method of Klinkhamer.[2]

The media selected for the isolation and enumeration of specific groups of human and marmoset oral microorganisms are shown in Fig. 1. Except for a few modifications, these media have been used quite extensively by several investigators.[3-14]

2. Hypobaric Pressure Chamber for Marmoset Studies.

Two plastic isolators were built to house two marmosets each in separate compartments in an atmosphere of 70 per cent oxygen and 30 per cent nitrogen at 5 PSI. Chamber atmosphere was controlled by the continuous recirculation of the chamber gases sequentially through canisters of chemical scrubbers to

a/Colab Laboratories, Inc., Chicago Heights, Illinois.

remove such impurities as CO_2, NH_3, H_2S, and organic contaminants from animal wastes. Oxygen was supplied automatically to the chamber as needed to maintain a partial pressure of 177 mm Hg. This was achieved by a light passing through a mercury manometer to energize a photocell and activate an oxygen valve whenever negative pressure within the chamber increased. The fabrication and operation of these units were described in the first annual NASA Report.[1] The recirculation unit and oxygen control system recently have been modified.

Initially the recirculation unit was fabricated of stainless steel. Although the system operated quite satisfactorily, the stainless steel was heavy and costly. A simpler, lighter and less expensive recirculation unit was built from transparent acrylic plastic at the University of Texas Dental Science Institute (Fig. 2 and 3). Since the unit contains transparent canisters the need to renew chemical scrubbers can now be detected visually (Fig. 4a).

The oxygen controller was modified by replacing the photocell device with nichrome wire pins in one column of the manometer to enable mercury to complete an electrical circuit with a specific increase of negative chamber pressure (Fig. 4c). The resulting electrical impulse was then amplified and relayed to a solenoid controlled oxygen valve.

The modified oxygen valve (Fig. 4d) consisted of a teflon plunger (2-1/8" long, 5/8" diameter) encased in two sections of 2" diameter acrylic plastic. The two sections of the housing were screwed together (1-1/4" threads) to enhance plunger alignment and sealed by an "O" ring. Two additional "O" rings were seated on the plunger (one near the end and the other near the center) to effect seals to chamber and oxygen supply ports. Upon activation of the solenoid by the oxygen controller, the plunger retracted from its closed position to allow oxygen from the supply tank to pass through the valve to the chamber. The compression spring attached to the solenoid and the plunger recoiled to close the valve upon solenoid deactivation (pressure readjustment by replenished O_2).

3. Oral Microbiologic Comparisons of Humans Under Normal and Unusual Circumstances.

Preliminary quantitative evaluations of specific oral microorganisms have been carried out on normal (noncancer and apparently healthy) individuals employed at the Dental Science Institute and cancer patients undergoing antileukemic therapy at the University of Texas M. D. Anderson Hospital and Tumor Institute. The cancer group consisted of patients confined to a conventional hospital environment and patients isolated in protected environments (sterile life island and laminar air flow units). The conventionally hospitalized patients received antibiotics only when needed to counteract infection; patients in protected environments were given an intensive antibiotic prophylactic regimen prior to isolation. It was believed that the microbiologic data derived from these subjects might reflect oral microbial changes similar to those occurring in environmental isolation of extended space flights.

Protected environmental units and prophylactic antibiotic regimens have been developed for patients undergoing intensive cancer chemotherapy.[15,16] The Life Island consists of a bed enclosed in a plastic tent. Air circulating in the unit is pumped through high efficiency filters capable of eliminating more than 99.97 per cent of the particles larger than 0.3 microns in diameter. When the tent is maximally inflated there is sufficient space for the patient to stand or sit beside the bed. Procedures are performed through plastic sleeves on the sides of the tent. All items placed in the unit are sterilized and passed in through locks equipped with ultraviolet light for surface decontamination. Items being removed from the unit also pass through the locks. A Laminar Air Flow Unit consists of a room with built-in high efficiency particulate air filters.[17-19] Air circulates through the room in a horizontal direction at a velocity of 90 ft/min and returns through a plenum in the ceiling. Personnel enter the unit attired in sterile cap, mask, gloves, gown, and boots. Before patient entry the units are chemically sterilized by fogging procedures. After patient entry, surfaces of the units are kept sterile with frequent applications of germicidal agents.

Patients subjected to the sterile environment undergo a four-day preparation. Axillary, pubic, and head hair are cut. Toenails and fingernails are clipped and cleaned with an orange stick and brush. The skin and hair are scrubbed twice daily with germicidal soaps. The teeth are brushed after each meal. The patient is provided with sterile bedclothes, linens, water, and food. Antibiotic prophylaxis is initiated during the four-day preparation period and is continued at four-hour intervals until the patient is removed from the unit. The patient receives topical application of mixtures of vancomycin, humatin, polymyxin B, nystatin, amphotericin B, neomycin, and bacitracin. Mixtures of these antibiotics are administered as nonabsorbable oral suspensions, nasal sprays, and ear drops and ointments.

Oral specimens from noncancer individuals were collected at 7:00 a.m. (or immediately upon arising) and at 10:00 a.m. semiweekly for a minimum of four weeks prior to any change in personal oral hygiene routine. Oral samples from cancer patients were collected, in most instances, only at 7:00 a.m. prior to oral cleansing, medication or breakfast. Some cancer patients will be sampled additionally at 10:00 a.m. until an optimum sampling period is established.

The cancer patients were sampled semiweekly before, during, and after exposure to experimental variables (antileukemic therapy, antibiotic therapy, type of environment, oral health regimen). Patient sampling was initiated upon hospital admission and diagnosis and was continued until termination of the hospital course.

4. Influence of Oral Hygiene Procedures on the Oral Microflora and of the Space Diets on Dental Plaque Accumulations.

Baseline counts characteristic of the oral flora and clinical evaluations of plaque score, gingival health index and the general state of oral health are being made in healthy volunteers according to the methods of Quigley and Hein.[20] The oral hygiene routine is reviewed and modified according to clinical and microbiologic findings. After an oral prophylaxis and two weeks of prescribed oral hygiene procedures, the individual is reexamined clinically and microbiologically to evaluate the effectiveness of the oral hygiene procedures. Any intensive oral hygiene procedures which will be prescribed for the cancer patients are being evaluated first in the normal subjects. Some of the methods under consideration are the use of bacterial inhibitors applied with irrigation devices and/or in ointments maintained *in situ* with mouth guards. The most effective and practical oral hygiene methods derived from these investigations will be explored for possible use in persons exposed to simulated or actual manned spacecraft flights.

Since toothbrushes will be used and stored for extended periods during isolation and in land-based space simulators and in eventual extended space flights, toothbrushes with either natural or nylon bristles have been tested for retention of viable microorganisms. After use, the bristles were removed aseptically to provide specimens for dilution and plate counts of residual microorganisms. The number of organisms recovered after various periods of storage (6 to 24 hours) were compared to counts obtained immediately after brushing.

The diets used in the Apollo 11 flight were analyzed for carbohydrate content. Special attention was given to the concentrations of sucrose and its potential for causing undesirable dental plaque accumulation. After determining the concentration of utilizable carbohydrate in the diets, extracted human teeth were exposed to extracts of the diets and dextranproducing microorganisms for assessment of the dental plaque producing potential.

RESULTS

1. Microbiologic Analyses of Different Oral Samples From Human and Marmoset.

Quantitative comparisons of the total aerobes and anaerobes recovered from four types of oral specimens collected semiweekly for one month from a healthy human subject showed an acceptable degree of reproducibility (Fig. 5). The counts from the 7:00 a.m. stimulated saliva and oral swabs were five to ten times higher than those from comparable specimens collected at 10:00 a.m. Counts from the 7:00 a.m. samples were more variable, however, than those from 10:00 a.m. samples; i.e., anaerobes from stimulated saliva at 7:00 a.m. $= \overline{X}$ of $1.2 \pm 2.9 \times 10^9$ v.s. $\overline{X}$ of $1.2 \pm 0.5 \times 10^8$ at 10:00 a.m. (Fig. 5).

The number of organisms recovered at 7:00 a.m. from a loop of residual saliva was approximately equal to those from the 10:00 a.m. samples, but again were quantitatively more variable: anaerobes at 7:00 a.m. $= \overline{X}$ of $6.8 \pm 11.9 \times 10^3$; anaerobes at 10:00 a.m. $= \overline{X}$ of $4.4 \pm 2.2 \times 10^3$ (Fig. 5).

Counts of 30-second oral mucus samples collected at 10:00 a.m. were about one $\log_{10}$ less than comparable counts obtained from 10:00 a.m. stimulated saliva. The reproducibility was about equal: anaerobes from mucus sample #9, $\overline{X}$ of $1.7 \pm 0.8 \times 10^7$; anaerobes from 10:00 a.m. stimulated saliva, $\overline{X}$ of $1.2 \pm 0.5 \times 10^8$. There was no significant difference in the quantity and reproducibility of counts between the 8th and 9th sequential mucus samples (Fig. 5).

The means and relative standard deviations of eight semiweekly counts of all the microbial categories enumerated from stimulated saliva collected at 7:00 a.m. and 10:00 a.m., and from oral mucus sample #9 are compared in Fig. 6. Although stimulated saliva collected at 7:00 a.m. provided the highest categorical counts (five to ten times), stimulated saliva collected at 10:00 a.m. provided more reproducible counts except for counts of streptococci.

The number of organisms counted within each specific category usually remained proportionally similar regardless of type of sample or time of collection with the exceptions of consistently higher counts of staphylococci and enteric organisms in 7:00 a.m. stimulated saliva.

Comparisons of the numbers of total aerobes, anaerobes and streptococci recovered from loop collected saliva of a normal human subject and two marmosets (Fig. 7a) demonstrated that the quantity and variability of the microbes were similar in both species. This was also true of the predominate organisms recovered from oral swabs (Fig. 7a) and endodontic paper points (Fig. 7b).

Although wire loops and paper points provided simple and relatively reproducible sampling methods, the specimens were too small to permit recovery of the categories of microorganisms which occur in low numbers. The oral swabs provided a larger sample and therefore were much more suitable for enumerating the groups of microorganisms occurring in small numbers. Swabs were weighed before and after use in an attempt to quantitate accurately the oral swab samples. Swab weights were too variable, however, due to the fiber loss to permit the counts to be expressed on a weight basis. Consequently they were recorded as counts per unit volume of swab suspension.

Counts of all the specific microbial categories enumerated from oral swab specimens from a normal human and a marmoset are compared in Fig. 8a and b. Counts from stimulated human saliva served as a reference. The most numerous specific group of oral microorganisms in the human was *Streptococcus sp.*, while *Neisseria sp.* predominated in the marmoset. Further contrast between the two animal species were the absence of *Streptococcus salivarius*, *Lactobacillus* and *Mycoplasma*, and the rare recovery of *Veillonella* and *Candida* species in the marmoset. Most other categories of oral microorganisms, however, were generally indistinguishable and numerically similar in man and marmoset (Fig. 8b).

2. Effects of Two-week Chamber Isolations on Marmosets.

The hypobaric chamber functioned without any insurmountable problems as described in the first annual NASA Report.[1] The chamber atmosphere was sampled at two hours and at 5, 7, and 14 days of operation. Readings with the Unico Gas Detector at two hours were identical to those of the laboratory atmosphere: 0.17 per cent CO_2 and no detectable hydrogen sulfide or ammonia. Readings after 5, 7, and 14 days were 0.35 per cent CO_2, 0.48 per cent CO_2, and 0.60 per cent CO_2, respectively with no detectable H_2S or NH_3.

After eliminating one of the dessicating agents ($CaCl_2$), the ophthalmic complications observed during the first three days of the first two-week isolation did not occur in any of the following two-week chamber isolations.

Weight losses noted during the first two-week isolation recurred during subsequent isolations except that the amount of weight loss decreased with each trial (Table 1). As in the first trial, weight was regained during the second week of chamber isolation.

Diarrhea consistently occurred during the second week of chamber isolation and persisted until the animals had been removed from the chamber for at least a week.

Calculus and plaque accumulations after each chamber isolation were comparable in amount to such accumulations at ambient conditions.

The chamber isolations were not associated with any significant changes in the number of microorganisms in loop collected saliva in either animal (Table 3 and 4).

Significant increases were found in total anaerobes, bacteroides and neisseria from the gingival sulcus of marmoset No. 1374 while a significant decrease occurred in enterics from oral swabs (Table 3). No significant quantitative changes were found in the gingival sulcus organisms from marmoset No. 1658, but there were significant increments in the number of total anaerobes and streptococci recovered from oral swabs of this animal (Table 4).

Counts from the oral swabs of both animals were graphed logarithmically in Fig. 9. The most numerous aerobic microorganisms identified was species of Neisseria followed by Streptococcus, Staphylococcus and enteric groups including species of Escherichia, Aerobacter, Proteus and Pseudomonas.

The most numerous identifiable group of anaerobes cultured from the marmosets were fusobacteria and bacteroides. Variable counts of veillonella were obtained from one of the animals and none from the other.

The animals responded to the four-week chamber isolation in a manner similar to the two-week chamber trials. Weight loss again was apparent after one week (Table 2). In contrast to the two-week chamber isolations, there was no weight regain until the animals were removed from the chamber.

Diarrhea again was observed during the second week of chamber isolation and persisted for about one week after the animals were removed from chamber isolation.

Counts from loop collected saliva, oral swabs and endodontic paper points from the gingival sulcus of marmosets isolated for four weeks in the chamber are presented in Fig. 10a, b, and c. The greatest numerical change in the oral microorganisms was an increase in salt tolerant staphylococci in each animal. The change occurred during the second week and persisted until the animals were removed from the chamber (Fig. 10a). In addition to elevated staphylococcal counts, one animal (No. 1658) had increased yeast and enteric counts by the fourth week of chamber isolation. Counts of the other microbial categories closely resembled those obtained during the two-week chamber tests.

3. Effect of Protected Environments and Antibiotic Regimens on the Oral Microflora.

Eight patients with acute leukemia were studied from the time they were hospitalized until treatment was terminated (3 to 16 weeks). Four were subjected to intensive antibiotic prophylaxis and protected environments (two to laminar air flow and two to life island units) and four were followed in the conventional hospital environment and received antibiotics only to combat infection. A summary of clinical histories is presented in Table 5.

Comparison of a conventionally hospitalized leukemia patient to a normal (noncancer) subject showed a quantitatively similar oral flora (Fig. 11). However, numerical differences were found in fusobacteria and candida and in the types of enteric organisms.

The fusobacteria and candida counts were unusually low in this leukemic patient compared to other leukemic patients and to normal subjects. The enteric category was comprised primarily of coliform bacteria in the hospital patients and Alcaligenes or Proteus in the normal individuals.

Comparisons of the predominate microflora of stimulated saliva from a control patient (conventionally hospitalized) and two test patients (subjected to antibiotic prophylaxis and protected environments) are shown in Fig. 12. Counts of the predominate organisms remained relatively constant in the control patient and decreased from levels of 10^8/ml saliva to levels of 10^5 to 10^7 in the test patients. There were no apparent differences in counts between patients confined to life island units and laminar air flow rooms (Fig. 12b).

The effects of intensive antibiotic prophylaxis and protected environments on specific types of oral microorganisms are represented by data from a patient treated in a laminar air flow room (Fig. 13). The oral microflora showed a general numerical decrease during therapy, except for an increase in candida and mycoplasma. Antibiotic treatment most effectively suppressed the fusobacteria, staphylococci, and enteric bacilli (included in the shaded areas in the graph in Fig. 13). Streptococci, bacteroides, and lactobacilli were relatively unaffected. Veillonella counts showed the greatest variability.

Two important aspects of the data in Fig. 13 are: (1) recovery of only Candida and Klebsiella from the throat and E. coli from the stool after the first week of treatment while relatively high levels of many different types of microbes were cultured from the oral cavity; and (2) the rapid increase of microorganisms upon discontinuance of antibiotics despite continued isolation in the protected environment.

The relation of oral microbial counts of clinical complications and antibiotic therapy in the control patients is shown in Fig. 14. Counts of microbial types which showed marked increases are plotted individually against body temperature, antibiotic received, and disease entity. Counts of organisms which decreased during therapy are included inthe shadowed areas of the graphs (Fig. 14 and 15).

The oral microorganisms which decreased most during intensive antibiotic prophylaxis in test patients (e.g., fusobacteria) also decreased when antibiotics were given for infection in the control patients. Most importantly, these data show a direct correlation of increased oral counts of the same or closely related microorganisms and those found to have caused fatal complications. This was vividly demonstrated by significant increases of E. coli in saliva four weeks prior to death of the patient from an E. coli septicemia (Fig. 14), and the linear increase of Candida albicans in saliva evident a few weeks prior to a patient's death from candidiasis (Fig. 15).

4. Effects of Oral Hygiene on Oral Microorganisms and Space Diets on Dental Plaque Accumulation.

Clinical evaluations before and after a prescribed oral hygiene regimen* of two subjects are presented in Table 6. Subject No. 1-LB had no inflammation and a relatively low plaque score which fell to zero within two weeks of treatment. The treatment also reduced the plaque and inflammation scores of Subject No. 2-SH whose initial values were much higher than those of patient 1-LB.

Means and standard deviations of oral microbial counts from subjects before and after two weeks of the prescribed oral hygiene regimen are shown in Tables 7 and 9. Statistical comparisons (t-test) of oral microbial counts revealed significant treatment related decreases in total streptococci, Streptococcus salivarius, fusobacteria, neisseria, lactobacilli, and mycoplasma in 7:00 a.m. and/or 10:00 a.m. stimulated saliva in one or both of the patients

*Prescribed oral hygiene regimen - prescribed method of toothbrushing to be performed one to three times daily, prescribed method of dental flossing (unwaxed dental floss) daily, and use of an irrigating device daily.

tested (Table 7). Significant post treatment increases were noted for neisseria in the 7:00 a.m. and for bacteroides and candida in the 10:00 a.m. stimulated saliva samples of subject No. 1-LB.

There were significant decreases in total aerobes and anaerobes, staphylococci and candida (10:00 a.m.) and lactobacilli (7:00 a.m.) oral swabs of subject No. 1-LB (Table 8). A greater range of statistically significant suppressions was found in Subject No. 2-SH. These involved total aerobes, total anaerobes, total streptococci, Streptococcus sp., Streptococcus salivarius, fusobacteria, veillonella, and staphylococci in 7:00 a.m. and/or 10:00 a.m. oral swabs (Table 8). There were no significant increases in any of the organisms recovered by the oral swab method.

No significant changes in counts in either direction were noted for the loop collected saliva (Table 9).

The number of microorganisms recovered from natural and nylon bristle toothbrushes following storage after use are compared in Table 8. Residual viable microorganisms from natural bristles declined significantly with 12 hours of storage. Significantly fewer microorganisms were cultured from the natural bristles than from nylon bristles after 24 hours storage. The levels of culturable microorganisms from the two types of toothbrushes at various storage intervals are presented graphically in Fig. 16.

The carbohydrate content of infusions* prepared from the daily diets used

*Infusions - all the dietary items of a given day were mixed together and rehydrated, infused in a 50°C water bath for one hour, boiled for ten minutes and filtered through cellulose until a clear infusate was obtained.

in the Apollo 11 flight was determined by the phenol-sulfuric acid method of Dubois et al.[21] The carbohydrate levels averaged 56.3 per cent per daily diet.

Aliquots from each infusion were diluted with distilled water to a concentration of approximately 5 per cent carbohydrate. The pH was adjusted to 7.0 and tubes containing 15 ml of "medium" were autoclaved and inoculated with a "cariogenic" streptococcus.* The ability of the infusion to support bacterial growth was evidenced by the acidity produced by the test organism in the infusions after 48 hours incubation at 37°C: Day 1 diet - pH 4.2, 0.02N; day 2 diet - pH 3.9, 0.03N; day 3 diet - pH 4.1, 0.03N; and day 4 diet - pH 3.9, 0.02N. The pH and normality produced in a standard culture medium[22] in 48 hours was 4.4, 0.05N.

The plaque forming potential of the infusions was tested _in vitro_ with extracted human teeth mounted in a coupling of rubber and glass tubing (Fig. 17a). After sterilization in ethylene oxide, the mounted teeth were exposed to 15 ml aliquots of the infusions inoculated with "cariogenic" streptococci (SL-1). All teeth were transferred daily until plaque accumulation on the teeth was sufficient to enable comparisons between teeth exposed to dietary infusions and those exposed to the regular culture medium.

Preliminary results are represented by six-day plaque accumulations of a tooth exposed to day 4 diet infusion and a tooth exposed to the regular

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*"Cariogenic" streptococcus - _Streptococcus_ _mutans_ strain SL-1 obtained from the National Institute of Dental Research, Bethesda, Maryland.

culture medium in Fig. 17.

DISCUSSION

The microbiologic analyses of oral specimens indicate that midmorning samples (samples collected at 10:00 a.m. or after the subject has established his daily routine) may be preferable to early morning samples (samples collected at 7:00 a.m. or immediately upon arising). Although the early morning sample provides higher counts of oral microorganisms, the mid-morning samples are more reproducible and therefore preferable for detecting numerical changes in the oral microbial populations.

The mode of sampling (e.g., wire loops, oral swabs, paper points, oral mucus or stimulated saliva) depends upon experimental objectives, environmental circumstances, and specimen availability. If a census of predominate salivary microorganisms is desired, a wire loop would be a practical and adequate method sampling for humans or marmosets regardless of environmental restriction or the condition of the subject; i.e., a comatose patient. Stimulated saliva or oral swabs are best if a census of microorganisms occurring in relatively small numbers is desired. Oral mucus samples are an appropriate method of sampling for supplementing the microbiologic data with counts of orogranulocytes (OMR) or a plaque precursing index (PPI).

The fact that certain oral microorganisms are commonly recovered from human saliva and not from the oral cavity of marmosets may be partly explained by the dry, low carbohydrate diet used in the chamber studies. This supposition is currently being investigated by sampling marmosets on a moist and relatively high carbohydrate diet.

A relatively light-weight, inexpensive chamber is suitable for studying the effects of space-simulated environments on the oral health of marmosets. No serious deleterious effects were observed in two marmosets after four two-week and one four-week chamber isolations. A transitory eye involvement which occurred only during the first two-week test is believed to have been caused by excessive dryness of the atmosphere. A rather excessive weight loss, particularly during the first chamber isolation, may be partially attributed to an unfamiliar environment, incomplete adjustment to food and water containers, and/or discomfort in an excessively dry atmosphere. This belief is based upon the decreased weight loss noted during repeated and prolonged exposures. The repeated problem of diarrhea during the second week of each chamber isolation is presently unexplained.

The importance of the marmoset oral microbial changes is presently obscured by the quantitative variability within specific categories of microorganisms in the same animal and the inconsistency of counts of certain groups of microorganisms between different animals. The significance of these changes awaits confirmation and substantiation.

The significance of the data obtained from leukemic patients is two-fold. First, the data emphasizes the importance of the oral cavity in reflecting local or systemic disease and stressful situations caused by certain indigenous or transient microorganisms. Second, the mouth may act as a reservoir for potentially pathogenic organisms whose increase or persistence may be important for the diagnosis and/or prediction of intraoral and extraoral pathosis and as a guide in antibiotic therapy.

The intensive antibiotic prophylactic regimen and protected environment were effective in eradicating organisms from all body orifices except the oral cavity. The lack of response of oral microorganisms to these treatments indicated the need for the use of different types of antibiotics or different methods of administering the antibiotics now prescribed. Also, a different approach to oral hygiene seems necessary to effectively control this reservoir of potential pathogens since the protected environment per se had little influence on these organisms.

Two weeks of a prescribed oral hygiene regimen in two individuals effectively reduced dental plaque and gingival inflammation. Concurrent microbial reductions varied with specific categories of microorganisms and were somewhat inconsistent between patients. Part of this variability might be eliminated by prolongation of treatment. Dramatic and consistent microbial reduction will probably require a more intensive oral hygiene regimen. This might be accomplished by adding microbial inhibitors to the oral hygiene procedures used in this study.

The decreased microbial recovery with increased storage time of used toothbrushes apparently was due to drying of the bristles. Toothbrushes with natural bristles dried faster and had lower levels of residual microorganisms with time of storage than those with nylon bristles.

Infusions of space diets supported the growth of a "cariogenic" streptococcus and the subsequent production of dextranous plaques on extracted human teeth *in vitro*. Since sucrose is required for plaque formation, glucose or some other carbohydrate could be substituted for sucrose to reduce the plaque producing potential of the space diets.

SUMMARY

Midmorning specimens appear to be preferable to early morning specimens for detecting quantitative changes in the oral microflora. Preference of the type of oral specimen; e.g., stimulated saliva, oral swabs, calibrated wire loop collection of residual saliva, etc., depends on the experimental objectives, environmental circumstances and specimen availability. Each showed a proportional similarity in numbers of specific organisms irrespective of the time of collection.

The oral microorganisms of man and marmoset were for the most part qualitatively similar. The dissimilarities were essentially quantitative with streptococci predominating in man and neisseria in the marmoset. Another notable difference was the absence of polysaccharide producing streptococci and lactobacilli in the two marmosets studied.

The hypobaric chamber functioned satisfactorily during the two-week and four-week trial periods. Diarrhea and weight loss continued to be a persistent problem associated with chamber isolation. Significant increments of total anaerobes, bacteroides, neisseria, and streptococci occurred in the animals exposed to two-week chamber isolations. The greatest numerical change in the oral microflora of animals isolated for four weeks was an

increase in staphylococci.

Microbiologic data from leukemic patients indicated that intensive antibiotic prophylactic regimens and protected environments were effective in eradicating organisms from all body orifices except the oral cavity. The data also demonstrated the importance of the oral flora in reflecting local or systemic disease and stressful situations. It was found that the mouth may act as a reservoir for potentially pathogenic organisms. An increase or persistence of such organisms may be diagnostic and/or predictive of intraoral and extraoral pathosis and could serve as a guide for appropriate antibiotic therapy.

Two weeks of a prescribed oral hygienic regimen effectively reduced dental plaque and gingival inflammation with variable reductions in the specific categories of oral microorganisms.

The number of microorganisms recovered from used toothbrushes diminished with time. The microbial decreases were associated with bristle dryness.

Infusions of space diets supported bacterial growth and provided the necessary substrate for dextranous plaque accumulation on extracted human teeth *in vitro.*

REFERENCES

1. Brown, L.R.; Wheatcroft, M.G.; and Allen, S.: Annual Report - National Aeronautics and Space Administration: Contract NAS 9 8200, June 1969.

2. Klinkhamer, J.M.: Quantitative Evaluations of Gingivitis and Periodontal Disease. I - The Orogranulocytic Migratory Rate, Periodontics, 6:5, 207-211, 1968.

3. Rogosa, M.; Mitchell, J.A.; and Wiseman, R.F.: A Selective Medium for the Isolation and Enumeration of Oral Lactobacilli, J. Dent Res, 30:682-689, 1951.

4. Rogosa, M.; Fitzgerald, R.J.; MacKintosh, M.E.; and Beaman, A.J.: Improved Medium for Selective Isolation of Veillonella, J. Bact, 76:455-456, 1958.

5. Omata, R.R.; and Disraely, M.N.: A Selective Medium for Oral Fusobacteria, J. Bact, 72:677-680, 1956.

6. Kraus, F.W.; and Gaston, C.: Individual Consistancy of Numbers Among the Oral Flora, J. Bact, 71:703-707, 1956.

7. Richardson, R.L.; and Jones, M.: Bacteriologic Census of Human Saliva, J. Dent Res, 37:697-709, 1958.

8. Shklair, I.L.; Mazzarella, M.A.; Gutekunst, R.G.; and Kiggins, E.M.: Isolation and Incidence of Pleuropneumonia-like Organisms from the Human Oral Cavity, J. Bact, 83:785-788, 1962.

9. McCarthy, C.; Snyder, M.L.; and Parker, R.B.: The Indigenous Oral Flora of Man. I - The New-born to the 1-year-old Infant, Arch Oral Biol, 10:61-70, 1965.

10. Ritz, H.L.: Microbial Population Shifts in Developing Human Plaque, Arch Oral Biol, 12:1561-1568, 1967.

11. Gibbons, R.J.; and Mac Donald, J.B.: Hemin and Vitamin K Compounds as required Factors for the Cultivation of Certain Strains of *Bacteroides melaninogenicus*, J. Bact, 80:164-170, 1960.

12. Socransky, S.S.; Gibbons, R.J.; Dale, A.C.; Bortnick, L.; Rosenthal, E.; and MacDonald, J.B.: The Microbiota of the Gingival Crevice. I - Total Microscopic and Viable Counts of Specific Organisms, Arch Oral Biol, 8:275-280, 1963.

13. Sonnewirth, A.C.: The Clinical Microbiology of the Indigenous Gram-Negative Anerobes (Synopsis from oral presentation at the Clinical Microbiology Round Table, A.S.M. Meeting, Atlantic City, New Jersey, 1965 - Personal Communication).

14. Finegold, S.M.; Miller, A.B.; and Posmak, D.J.: Further Studies on Selective Media for Bacteroides and other Anaerobes, Ernährungsforschung, X/2/3:517-528, 1965.

15. Levitan, A.A.; and Perry, S.: Infectious Complications of Chemotherapy in a Protected Environment, New Eng, J. Med, 276:881-886, 1967.

16. Bodey, G.P.; Hart, J.; and Freireich, E.J.: Prolonged Survival of a Leukemic Patient in a Protected Environment, Am J. Med Sci, 256:112, 1968.

17. Yates, G.; and Bodey, G.P.: Laminar Air for Cancer Patients, Contamination Control, pp. 20-26, 1968.

18. Bodey, G.P.; Hart, J.; Freireich, E.J.; and Frei, E. III: Studies of a Patient Isolator Unit and Prophylactic Antibiotics in Cancer Chemotherapy, Cancer, 22:1018-1026, 1968.

19. Bodey, G.P.: Laminar Air Flow Unit for Patients Undergoing Cancer Chemotherapy, Proc. 1968. Symposium of Gnotobiotics, Gnotobiology: Experimental and Clinical Aspects, In Germ-free Biology, Plenum Press, 1969.

20. Quigley, G.A.; and Hein, J.W.: Comparative Cleaning Efficiency of Manual Power Brushing, JADA, 65:26-29, 1962.

21. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; and Smith, F.: Colorimetric Method for Determination of Sugars and Related Substances, Anal Chem, 28:350-356, 1956.

22. Jordan, H.V.; Fitzgerald, R.J.; and Bowler, A.E.: Inhibition of Experimental Caries by Sodium Metabisulfite and Its Effect on the Growth and Metabolism of Selected Bacteria, J. Dent Res, 39:116-123, 1960.

TABLE 1

WEIGHTS (IN GRAMS) OF MARMOSETS
BEFORE, DURING AND AFTER TWO-WEEK CHAMBER ISOLATIONS

Test No.	Animal No.	Pre-chamber	In Chamber				Postchamber	
			1st week	Diff.	2nd Week	Diff.	1 Week	Diff.
I	1374	424	380	-44	405	-19	411	-13
	1658	410	353	-57	381	-29	393	-17
II	1374	427	404	-23	408	-19	412	-15
	1658	408	387	-21	391	-17	387	-21
III	1374	410	393	-17	397	-13	409	- 1
	1658	408	398	-10	403	- 5	402	- 6

TABLE 2

WEIGHTS (IN GRAMS) OF MARMOSETS
BEFORE, DURING AND AFTER A FOUR-WEEK CHAMBER ISOLATION

Animal No.	Pre-chamber	In Chamber								Postchamber	
		1st Wk.	Diff.	2nd Wk.	Diff.	3rd Wk.	Diff.	4th Wk.	Diff.	1 Wk.	Diff.
1374	421	397	-24	390	-31	397	-24	403	-18	407	-14
1658	419	404	-15	406	-13	412	- 7	408	-11	414	- 5

TABLE 3

ORAL MICROBIAL COUNTS FROM MARMOSET 1374
BEFORE, DURING AND AFTER TWO-WEEK CHAMBER ISOLATIONS

Specimens and Dilutions	Microbial Categories	Pre-chamber			In Chamber 1st Week		In Chamber 2nd Week		Post-chamber		
		$\overline{X}$	S.D.	N	$\overline{X}$	S.D.*	$\overline{X}$	S.D.*	$\overline{X}$	S.D.	N
Saliva Loop n X 10^5/ml saliva	Total anaerobes	281	242	25	148	147	28	12	274	400	12
	Total aerobes	169	160	23	87	66	28	28	97	190	12
	Neisseria	53	60	8	12	--	6	--	196	322	4
	Streptococci	36	44	23	36	14	1.4	0.8	52	150	12
Oral Swab n X 10^4/ml swab susp.	Total anaerobes	594	429	13	1240	--	530	--	598	404	7
	Total aerobes	328	166	15	271	--	683	--	178	269	8
	Neisseria	200	114	9	116	--	354	--	89	85	4
	Streptococci	49	31	15	92	--	420	--	33	20	8
	Fusobacteria	16	24	21	7	6	8	7	13	13	12
	Bacteroides	6	9	21	27	45	11	19	11	16	12
	Veillonella	0.02	0.05	21	0.02	0.04	0.001	0.002	0.01	0.03	12
	Staphylococci	0.02	0.03	21	0.001	0.001	0.01	0.02	0.02	0.04	12
	Enterics	0.02	0.03	21	0	0	0.003	0.002	0.002**	0.005	12
Gingiva Sulcus n X 10^3/ml paper point susp.	Total anaerobes	469	257	26	1180***	1133	231	32	339	132	12
	Total aerobes	169	187	26	94	63	61	27	83	73	12
	Neisseria	16	13	8	21	--	1	--	35**	12	4
	Fusobacteria	14	22	26	2	2	5	7	6	4	12
	Bacteroides	8	17	26	60**	104	14	25	14	25	12
	Streptococci	6	5	26	1	0.6	3	5	10	12	12

*Standard deviations calculated with an N of three.
**Significant changes from prechamber values at P < .05.
***Significant increase from prechamber value at P < .02.

TABLE 4

ORAL MICROBIAL COUNTS FROM MARMOSET 1658
BEFORE, DURING AND AFTER TWO-WEEK CHAMBER ISOLATIONS

Specimens and Dilutions	Microbial Categories	Pre-chamber			In Chamber 1st Week		In Chamber 2nd Week		Post-chamber		
		$\overline{X}$	S.D.	N	$\overline{X}$	S.D.*	$\overline{X}$	S.D.*	$\overline{X}$	S.D.	N
Saliva-Loop n X 10^5/ml Saliva	Total anaerobes	102	66	24	59	90	48	17	140	151	12
	Total aerobes	57	43	24	30	39	29	24	83	139	12
	Neisseria	33	28	8	4	--	26	--	19	20	4
	Streptococci	37	60	24	8	6	11	18	22	37	12
Oral Swab n X 10^4/ ml swab susp.	Total anaerobes	381	257	13	352	--	668	--	831**	625	7
	Total aerobes	156	128	13	179	--	116	--	210	218	8
	Neisseria	53	38	9	85	--	68	--	132	149	4
	Streptococci	8	6	13	4	--	12	--	18***	10	8
	Fusobacteria	8	13	20	3	6	7	6	19	26	12
	Bacteroides	3	7	20	0.8	0.9	0.9	1.6	6	9	12
	Staphylococci	0.06	0.12	20	0.01	0.01	0.03	0.02	0.03	0.03	12
	Enterics	0.03	0.05	20	0.03	0.03	0.02	0.02	0.1	0.2	12
Gingiva Sulcus n X 10^3/ml paper point susp.	Total anaerobes	351	328	24	493	411	436	702	395	322	12
	Total aerobes	110	118	24	118	92	235	230	116	171	12
	Neisseria	24	34	8	202	--	101	--	46	44	4
	Fusobacteria	25	37	24	37	55	40	53	10	7	12
	Streptococci	16	28	24	23	34	36	56	6	9	12
	Bacteroides	2	4	24	0.4	0.7	0	0	2	3	12

*Standard deviations calculated with an N of three.
**Significant changes from prechamber values at P $\leq$ .05.
***Significant increase from prechamber value at P $\leq$ .01.

TABLE 5 - PATIENT DESCRIPTION, DIAGNOSIS, TREATMENT AND HOSPITAL COURSE

Patient No. & Age Sample Period Diagnosis	Cancer Chemotherapy	Clinical Complications	Antibiotic Therapy	Hospital Course
C_1 25 yr., 2 mos Reticulum cell sarcoma with leukemic transformation.	l-asparaginase Cyto. arab.* Methyl GAG	Pseudomonas septicemia Pseudomonas cellulitis E. coli septicemia Pseudomonas cellulitis Pseudomonas septicemia and pneumonia Serratia septicemia	Carbenicillin Chloromycetin Cephaloglycin Gentamicin Keflin Kanamycin Polymyxin	Expired - Pneumonia & Septicemia (Pseudomonas)
C_2 62 yr., 2 mos Acute Granulocytic leukemia.	Cyto. arab. Cytoxan Vinicristine Prednisone	E. coli septicemia & Infectious myositis	Carbenicillin Keflin Polymyxin B Gentamicin	Expired - Infectious myositis & septicemia (E. coli).
C_3 55 yr., 3 wks Acute Undifferentiated Leukemia	Vincristine Prednisone Cyto. arab. Cytoxan	Disseminated candidiasis	Kanamycin Keflin Carbenicillin Polymyxin 5-Fluorocytosine	Expired - Disseminated Candidiasis (C. albicans)
C_4 15 yr., 1 mo Acute lymphocytic Leukemia	Cyto. arab.	Fever of undetermined origin Gingivitis	Carbenicillin Kanamycin	Discharged - Complete Remission
T_1 45 yr., 2 mos Acute Myelocytic Leukemia - Laminar Air	Cyto. arab. Cytoxan Vincristine Prednisone	None	Cloxacillin	Discharged - Repression
T_2 33 yr., 2 mos Acute Lymphocytic Leukemia Laminar Air	l-asparaginase Imidazole Carb** Cyto. arab. POMP	Generalized Herpes zoster	Carbenicillin BLP 1462 Gentamycin	Expired - Generalized Herpes zoster
T_3 18 yr., 4 mos Acute Lymphocytic Leukemia - Life Island	Cyto. arab. Cytoxan Vincristine Prednisone	Pharyngitis Fever of undetermined origin Paronychia & cellulitis of R. great toe Urinary Tract Infection - Enterococcus	Carbenicillin Kanamycin Tetracycline Keflin Polymyxin	Discharged - partial remission
T_4 18 yr., 2 mos Acute Myelogenous Leukemia - Life Island	Daunomycin Degg† Alg††	Serratia septicemia E. coli septicemia Sinusitis Serratia cellulitis & otitis externa Thrush	Amphotericin Gentamicin Amphicillin Carbenicillin Kanamycin Keflin	Expired - Septicemia (E. coli)

* Cytosine arabinoside ** Imidazole carboxamid traizine *** Prednisone, Oncovin, Methotrexate, P-6 Mercapopurine
† Deactivated equine gamma globulin †† Antileukocytic globulin

TABLE 6

CLINICAL EVALUATIONS BEFORE AND AFTER TWO WEEKS
OF A PRESCRIBED ORAL HYGIENE REGIMEN

Patient No.	Dental Plaque Scores*		Inflammation Index**	
	Before	After	Before	After
1-LB	1.30	0.00	0.00	0.00
2-SH	3.75	0.21	1.46	0.08

*Plaque scores can range from 0-6.
**Inflammation indices can range from 0-12.

TABLE 7

MEANS AND STANDARD DEVIATIONS OF MICROBIAL COUNTS FROM STIMULATED SALIVA OF SUBJECTS BEFORE AND AFTER A TWO-WEEK PRESCRIBED ORAL HYGIENE REGIMEN

Microbial Category	Dil.	Subject No. 1-LB				Subject No. 2-SH			
		7:00 a.m. Sample		10:00 a.m. Sample		7:00 a.m. Sample		10:00 a.m. Sample	
		Before	After	Before	After	Before	After	Before	After
Aerobes	$n \times 10^7$	58±35	37±12	10±5	17±7	85±113	21±13	9±7	4±4
Anaerobes		123±291	59±48	12±5	20±19	50±54	29±17	13±8	14±12
Total Strep		55±44	22±18	12±16	8±8	31±20	15±7	8±3	4±3++
Strep Species		51±44	22±18	9±13	8±8	29±21	15±7	6±3	4±3
Strep salivarius		4±2	0.3±0.3*	3±3	0.3±0.2++	2±3	0.1±0.06	2±1	0.1±0.1**
Bacteroides	$n \times 10^5$	248±331	122±140	9±11	160±167	320±253	790±1246	298±208	266±314
Fusobacteria		39±35	5±5++	2±2	4±4	25±23	45±59	43±40	84±116
Veillonella		584±452	151±234	125±93	225±227	38±88	6±11	67±106	4±6
Neisseria		12±10	134±93**	4±3	24±36	473±390	112±60	43±118	102±109
Salt tolerant staphylococci	$n \times 10^3$	8330±6221	7363±14,347	39±32	20±29	5±3	2±0.6+	2±1	0.7±0.5**
Lactobacilli		58±38	12±6**	23±15	5±2**	0	0	0	0
Candida		8±8	7±4	0.7±0.3	2.0±0.8**	0	0	0	0
Mycoplasm	$n \times 10^1$	0	0	0	0	2720±2970	1610±1420	6320±4690	2260±1710+
Enterics		3919±3872	5246±5550	10±13	18±22	0.4±0	0.7±2	0.2±0.3	0.2±0

*Significant Changes $P < 0.001$.
**Significant changes $P < 0.01$.
+Significant changes $P < 0.02$.
++Significant changes $P < 0.05$.

TABLE 8

MEANS AND STANDARD DEVIATIONS OF MICROBIAL COUNTS FROM ORAL SWABS OF SUBJECTS BEFORE AND AFTER A TWO-WEEK PRESCRIBED ORAL HYGIENE REGIMEN

Microbial Category	Dil.	Subject No. 1-LB				Subject No. 2-SH			
		7:00 a.m. Sample		10:00 a.m. Sample		7:00 a.m. Sample		10:00 a.m. Sample	
		Before	After	Before	After	Before	After	Before	After
Aerobes	$n \times 10^6$	102±118	68±34	82±63	13±19+	61±54	16±10++	14±17	2±1
Anaerobes		126±105	50±14	104±80	10±10**	81±41	24±29**	12±5	2±0.9*
Total Strep		51±22	52±45	48±49	10±18	80±56	16±12++	10±6	0.9±0.6**
Strep species		46±24	51±45	40±39	10±18	79±56	16±12+	9±7	0.8±0.6**
Strep salivarius		2±2	0.4±0.2	8±11	.03±.02	0.6±0.7	.06±.07++	0.7±0.5	.05±.07**
Bacteroides	$n \times 10^5$	14±23	25±24	5±7	7±6	35±38	9±6	6±4	4±2
Fusobacteria		0.8±0.9	0.4±0.4	0.5±0.5	0.2±0.3	9±16	1±0.9	5±4	0.8±0.4++
Veillonella		55±82	19±25	28±32	7±9	5±2	0.3±0.6*	1±1	0.1±0.3
Neisseria		3±3	13±15	2±1	1±2	146±152	15±20++	40±81	3±2
Salt tolerant staphylococci	$n \times 10^3$	5151±5851	3353±5990	19±18	3±5+	0.7±1	0.08±0.2	0.03±0.03	0
Lactobacilli		5±4	0.6±0.3+	5±8	.07±.09	0	0	0	0
Candida		3±4	1±0.6	0.4±0.4	.04±.05++	0	0	0	0
Mycoplasma	$n \times 10^1$	0	0	0	0	0	0	0	6±12
Enterics		5989±8244	15, 159±35, 596	41±105	1±2	0.06±0	0.1±0.3	0	0

*Significant changes P < 0.001.
**Significant changes P < 0.01.
+Significant changes P < 0.02.
++Significant changes P < 0.05.

TABLE 9

MEANS AND STANDEARD DEVIATIONS OF MICROBIAL COUNTS FROM LOOP SALIVA OF SUBJECTS BEFORE AND AFTER A TWO-WEEK PRESCRIBED ORAL HYGIENE REGIMEN

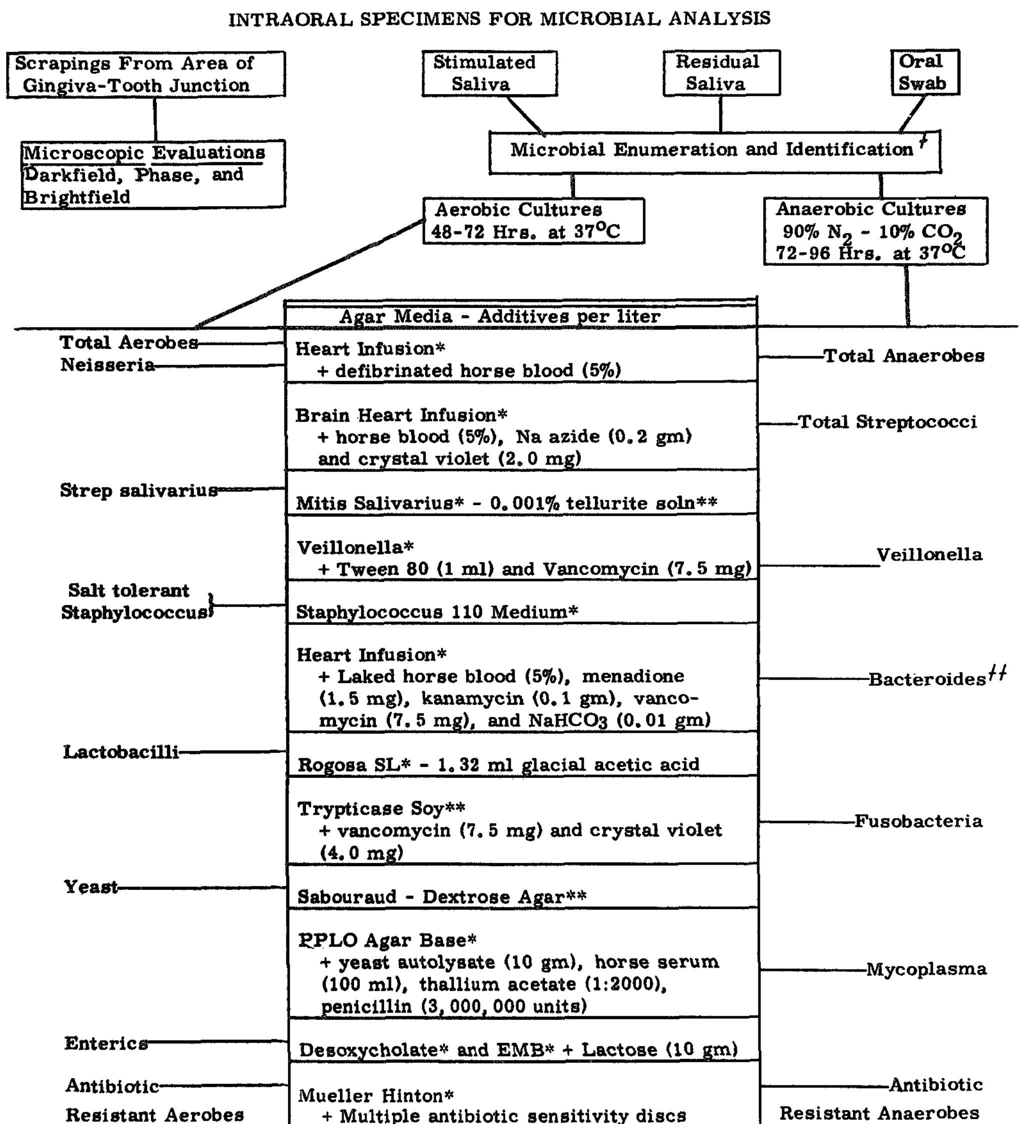
Microbial Category	Dil.	Subject No. 1-LB				Subject No. 2-SH			
		7:00 a.m. Sample		10:00 a.m. Sample		7:00 a.m. Sample		10:00 a.m. Sample	
		Before	After	Before	After	Before	After	Before	After
Aerobes	$n \times 10^2$	43 ± 60	21 ± 25	32 ± 22	34 ± 39	263 ± 150	522 ± 788	48 ± 34	38 ± 64
Anaerobes		68 ± 119	35 ± 46	44 ± 22	81 ± 63	767 ± 949	1459 ± 1855	156 ± 200	122 ± 200
Total Strep		49 ± 110	11 ± 19	14 ± 16	15 ± 35	607 ± 477	323 ± 389	33 ± 13	42 ± 79
Neisseria		0.8 ± 1.0	6 ± 7	0.4 ± 0.4	4 ± 5	3 ± 3	8 ± 17	0.01 ± 0.04	0.2 ± 0.3

TABLE 10

MEANS AND STANDARD DEVIATIONS OF MICROORGANISMS RECOVERED FROM TOOTHBRUSH BRISTLES

Storage Time (Hours)	N	Nylon Bristle $\overline{X}$		Nylon Bristle S.D.	Natural Bristle $\overline{X}$		Natural Bristle S.D.
0	11	2,323	±	2,641	2,122	±	1,597
6	11	1,585	±	1,146	1,279	±	1,822
12	11	1,495	±	1,174	204*	±	253
24	11	716	±	691	273**	±	512

*A significant decrease ($P < .01$) from 0 storage time of natural bristle brush and from 12 hour storage of the nylon bristle brush.
**A significant decrease ($P < .01$) from 0 storage time of natural bristle brush.



*Difco
**BBL

ǂStandard classification procedures for: predominating organisms, antibiotic resistant isolates, and all enteric and yeast isolates.
ǂǂBacteroides were incubated in Brewer jars in 95 per cent H_2 and 5 per cent CO_2.

Fig. 1. Procedure used for the isolation and enumeration of specific groups of human and marmoset oral microorganisms.

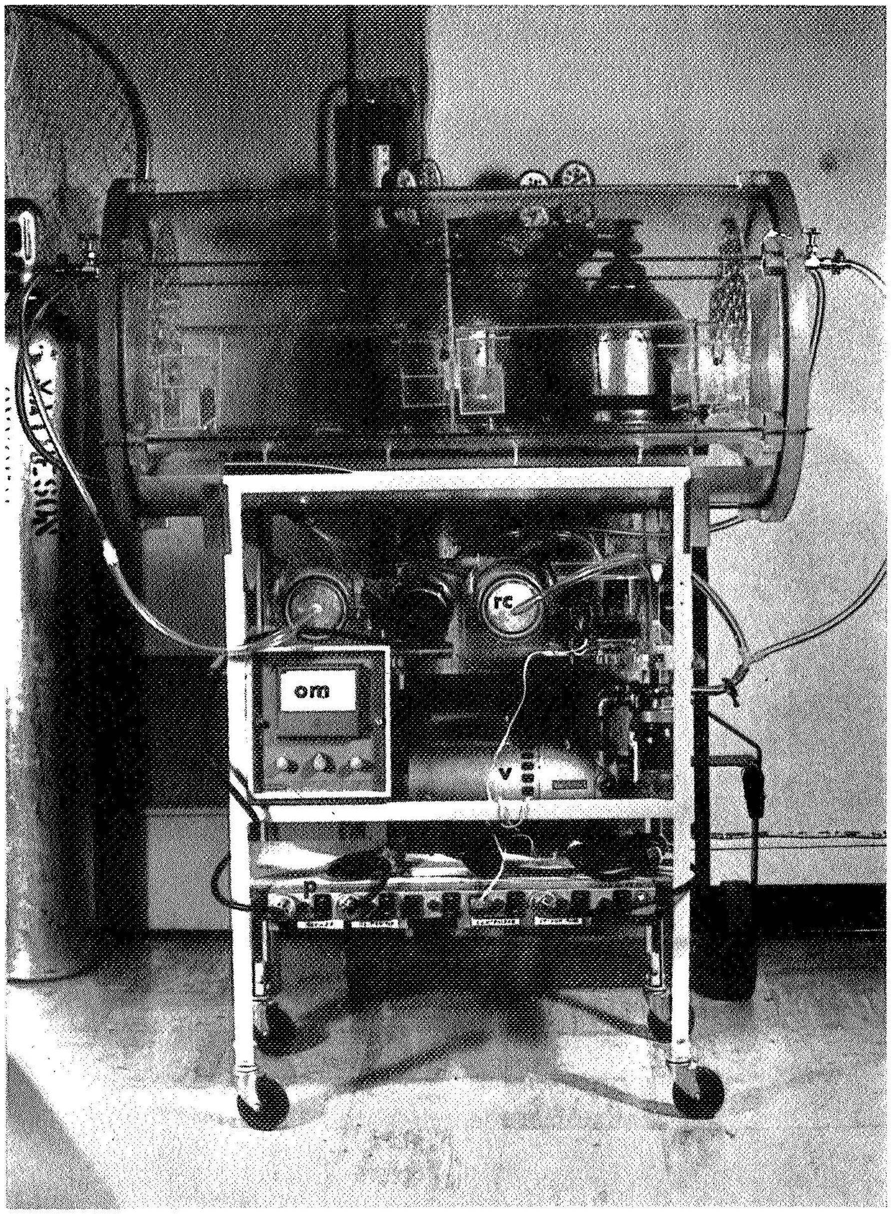


Fig. 2. Hypobaric pressure chamber with modified plastic recirculation system:

(rc) recirculation unit,
(om) oxygen monitor,
(v) vacuum pump,
(p) electric pilot strip.

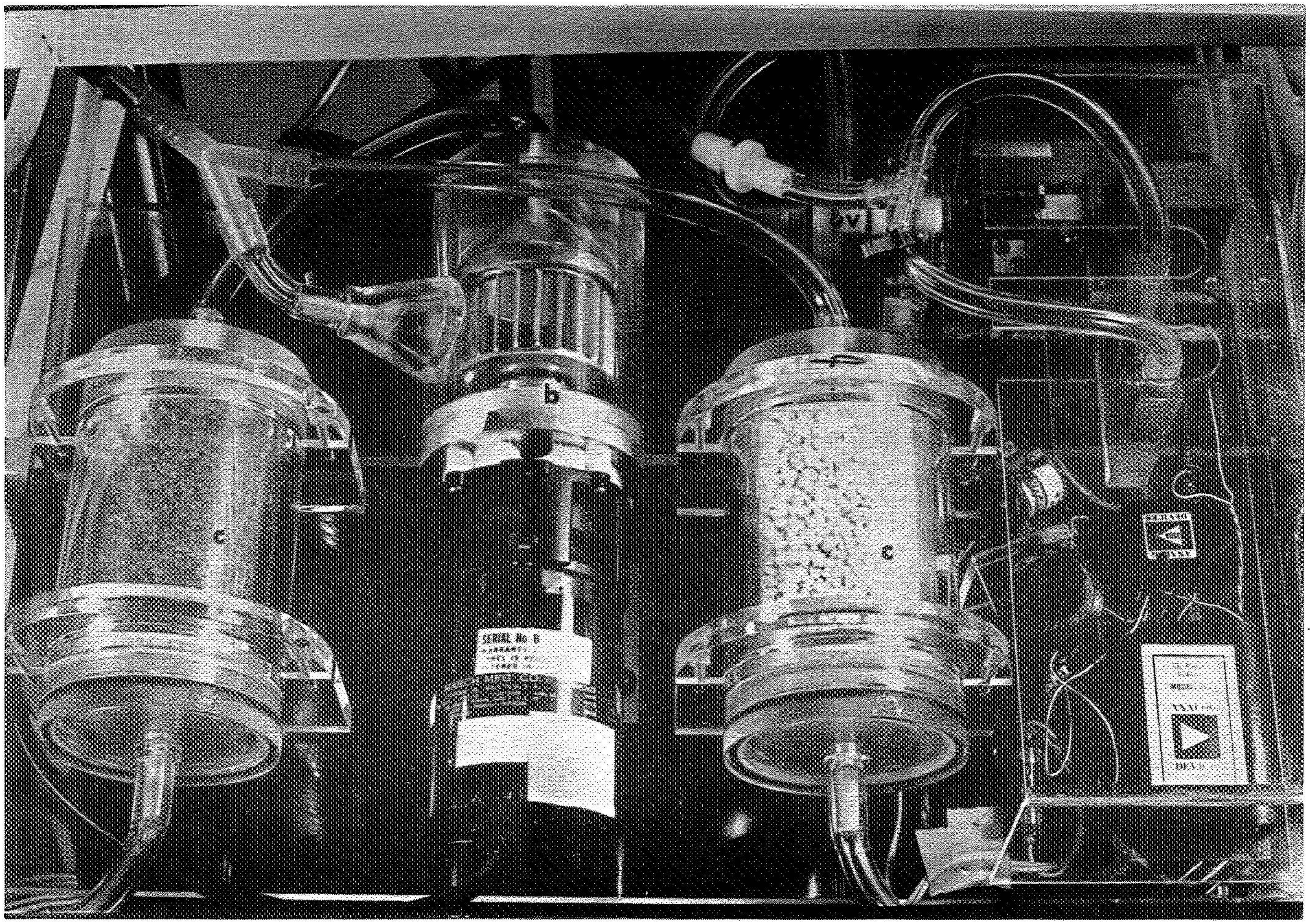


Fig. 3. Components of recirculation and oxygen control systems:

(c) canisters for chemical scrubbers,
(b) blower,
(oc) oxygen controller,
(ov) oxygen valve.

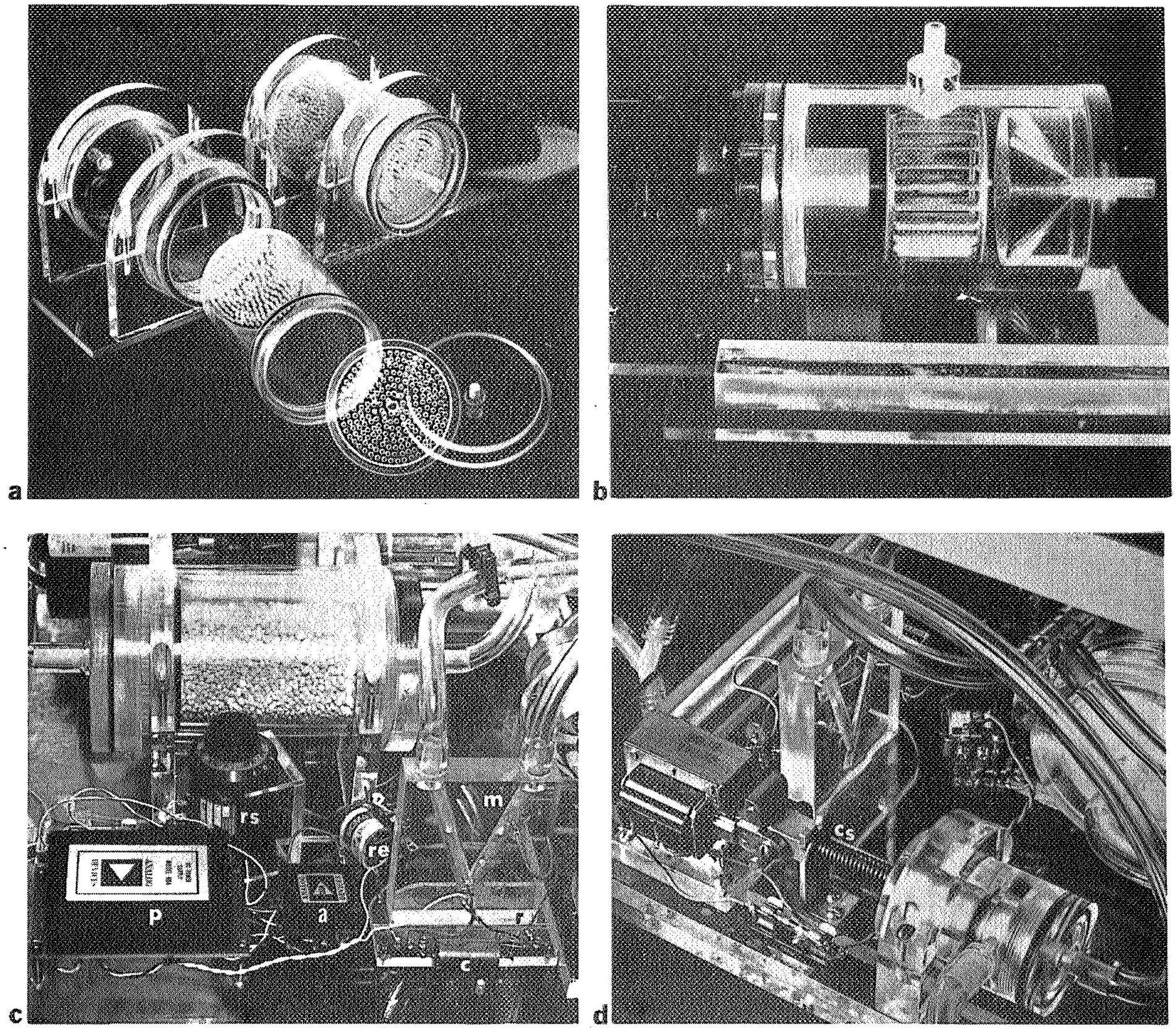


Fig. 4. Magnified view of specific units of the recirculation and oxygen control system: (a) parts of a scrubbing canister, (b) magnetically driven squirrel cage blower within an acrylic plastic case with a funnel-shaped inlet, (c) oxygen controller consisting of a "V" shaped mercury manometer [m] formed in acrylic plastic with nichrome wire pins in one column, a capacitor [c] and resistor [r] to produce an electrical lag, power supply [p], amplifier [a], relay [re], and rheostat [rs], (d) solenoid operated O_2 valve consisting of a compression spring [cs] and a teflon plunger sealed with "O" rings within an acrylic plastic case to allow or prevent passage of O_2 to pressure chamber.

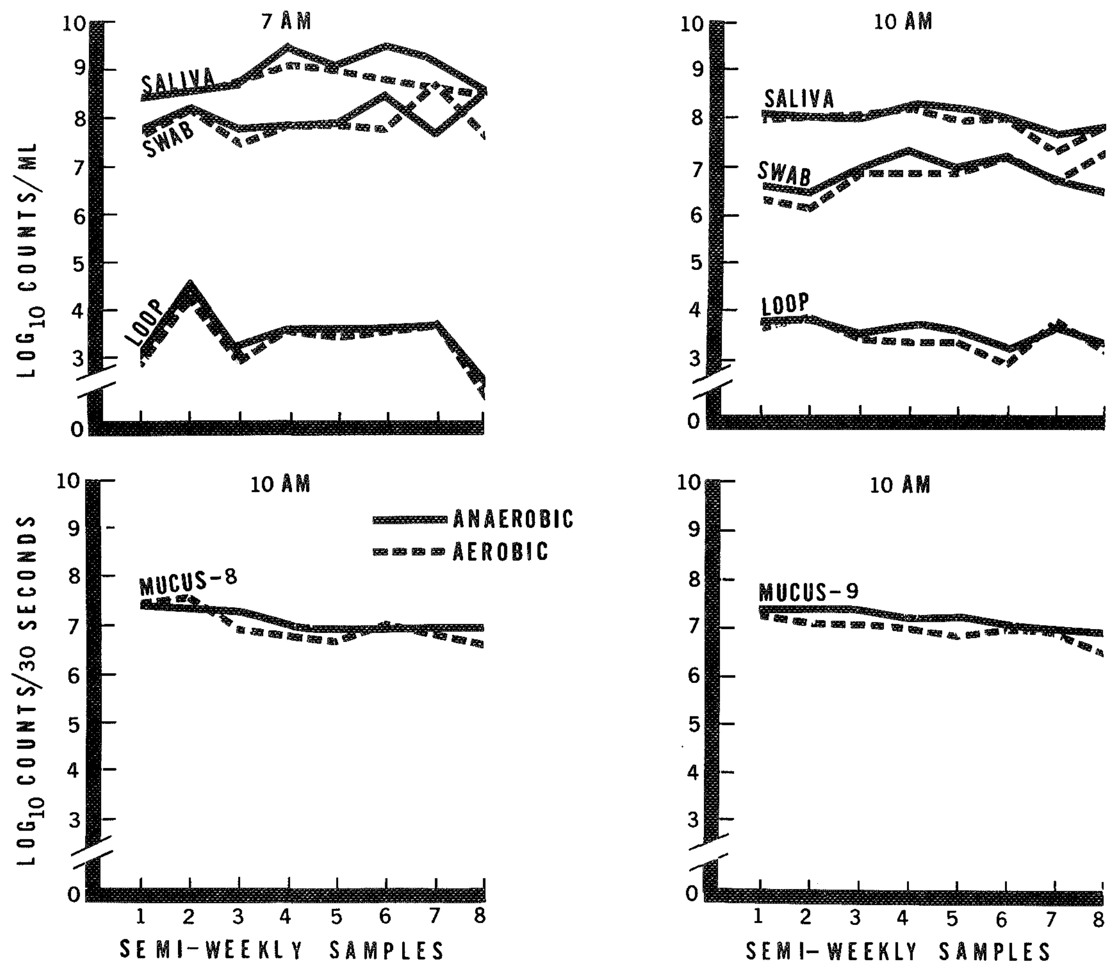


Fig. 5. Comparison of total aerobic and anaerobic counts from different types of oral specimens from a representative normal human subject.

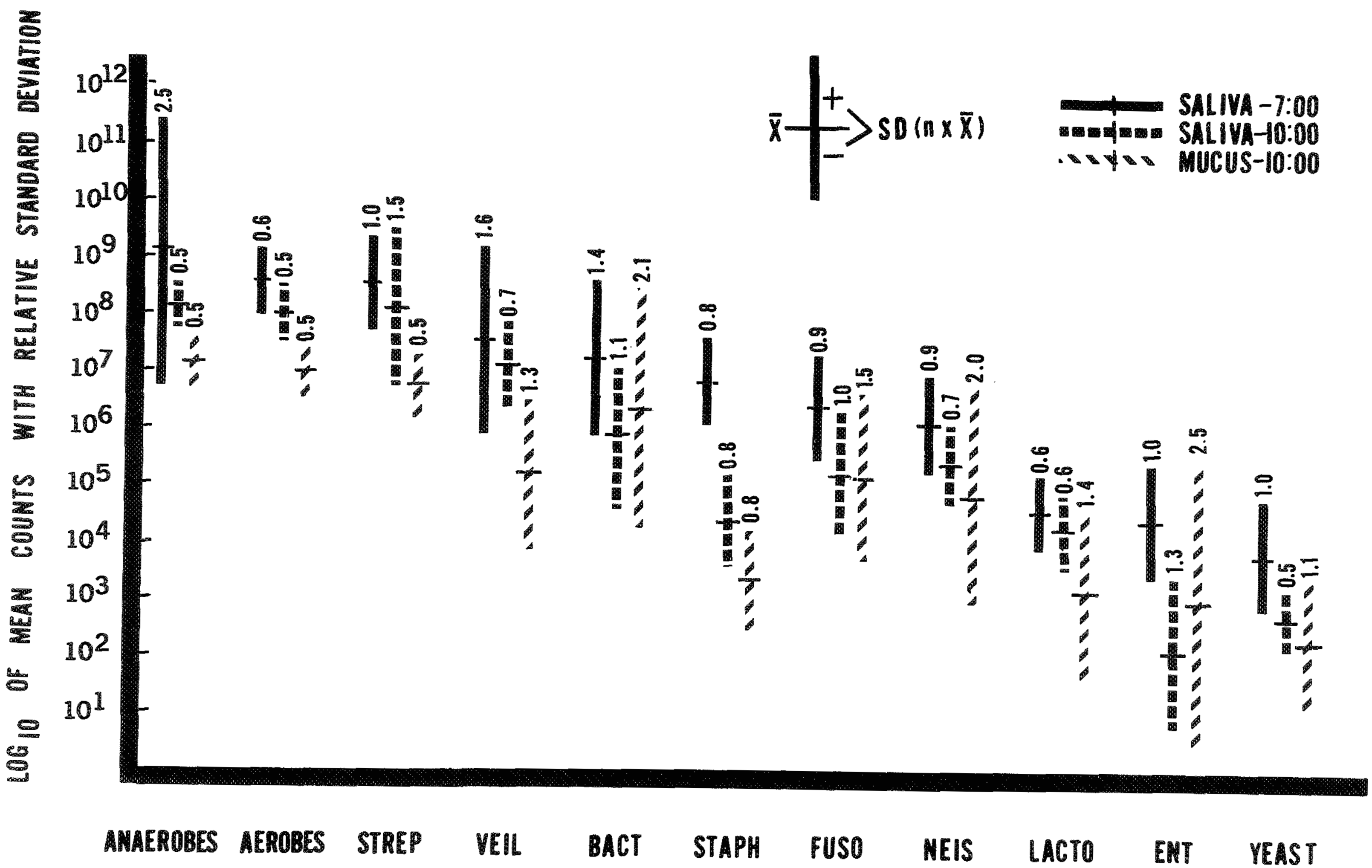


Fig. 6. Comparison of the means and relative standard deviations within microbial categories enumerated from different oral specimens from a representative normal human subject.

Abbreviations: STREP=streptococci, VEIL=veillonella, BAC=bacteroides, STAPH=staphylococci, FUSO=fusobacteria, NEIS=neisseria, LACTO=lactobacilli, ENT=enterics.

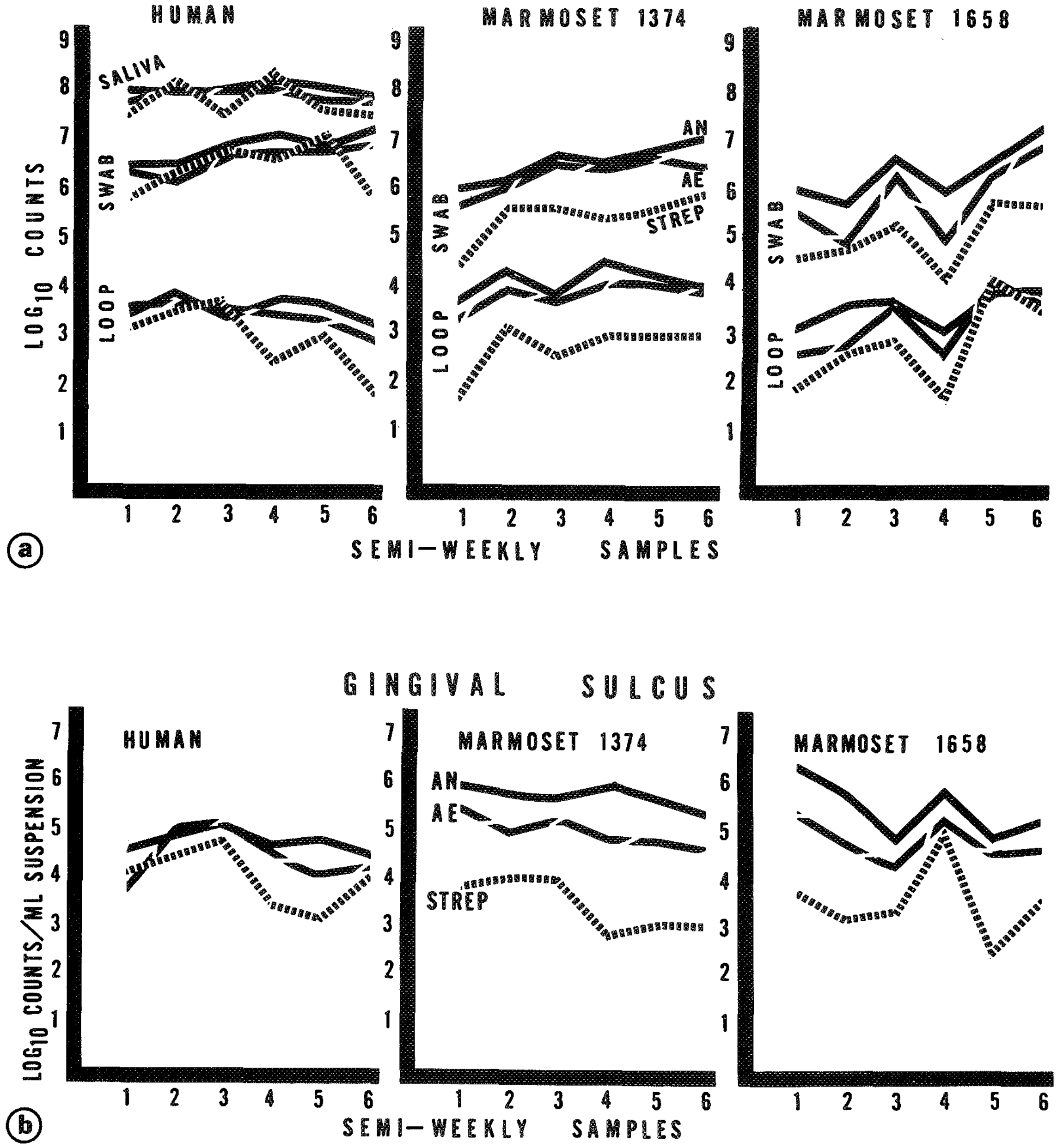


Fig. 7. A numerical comparison of predominate cultivable microorganisms in oral specimens collected from human and marmoset: (a) counts from oral swabs, stimulated, and loop collected saliva, (b) counts from the gingival sulcus.

Abbreviations: AN=anaerobes, AE=aerobes, STREP=streptococci.

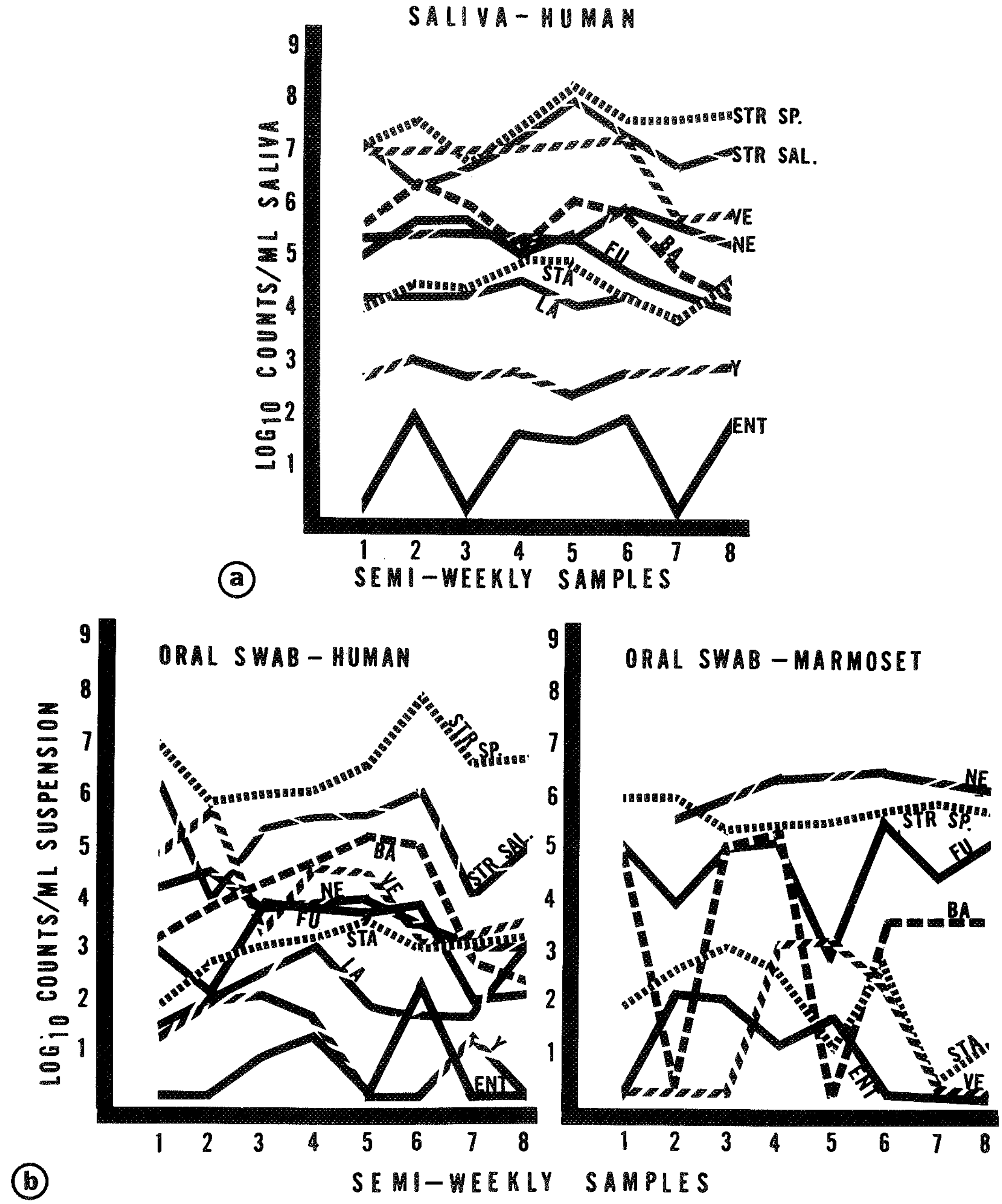


Fig. 8. Comparison counts of specific groups of oral microorganisms from human and marmoset, counts from: (a) stimulated human saliva used as a reference, (b) oral swabs from a representative normal human subject and marmoset.

Abbreviations: STR SP= Streptococcus sp., STR SAL=Streptococcus salivarius, VE=veillonella, NE=neisseria, BA=bacteroides, FU=fusobacteria, STA=staphylococci, LA=lactobacilli, Y=yeast, ENT=enterics.

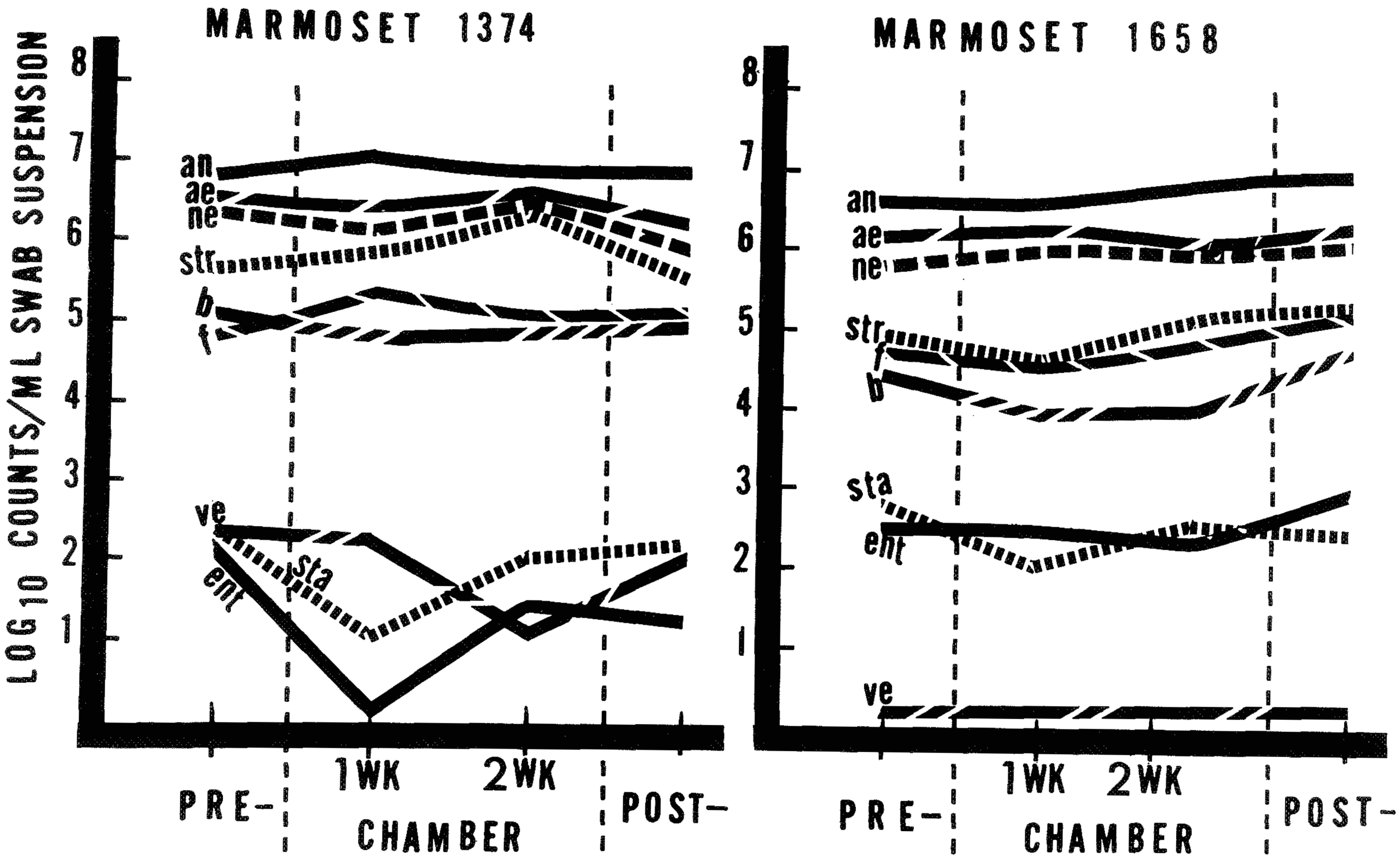


Fig. 9. Comparison of mean microbial counts from oral swabs of marmosets before, during and after two-week chamber isolations.

Abbreviations: AN=anaerobes, AE=aerobes, NE=neisseria, STR=streptococci, B=bacteroides, F=fusobacteria, VE=veillonella, STA-staphylococci, ENT=enterics.

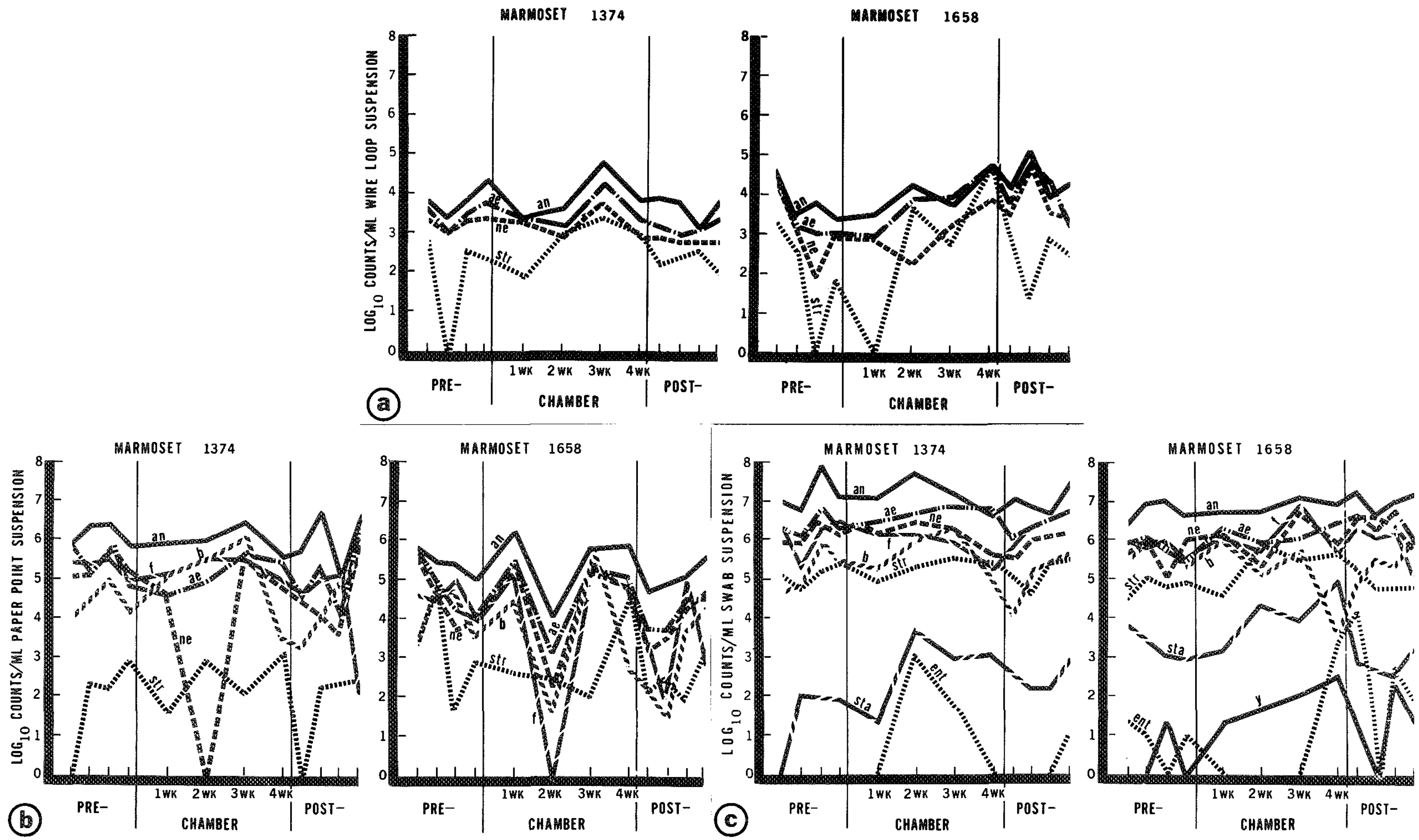


Fig. 10. Comparison of oral microbial counts from marmosets before, during and after a four-week chamber isolation, counts of: (a) predominate organisms from loop collected saliva, (b) predominate organisms from paper point suspensions, (c) specific organisms from oral swabs.

Abbreviations: an=anaerobes, ae=aerobes, ne=neisseria, str=streptococci, f=fusobacteria, b=bacteroides, sta=staphylococci, ent=enterics, y=yeast.

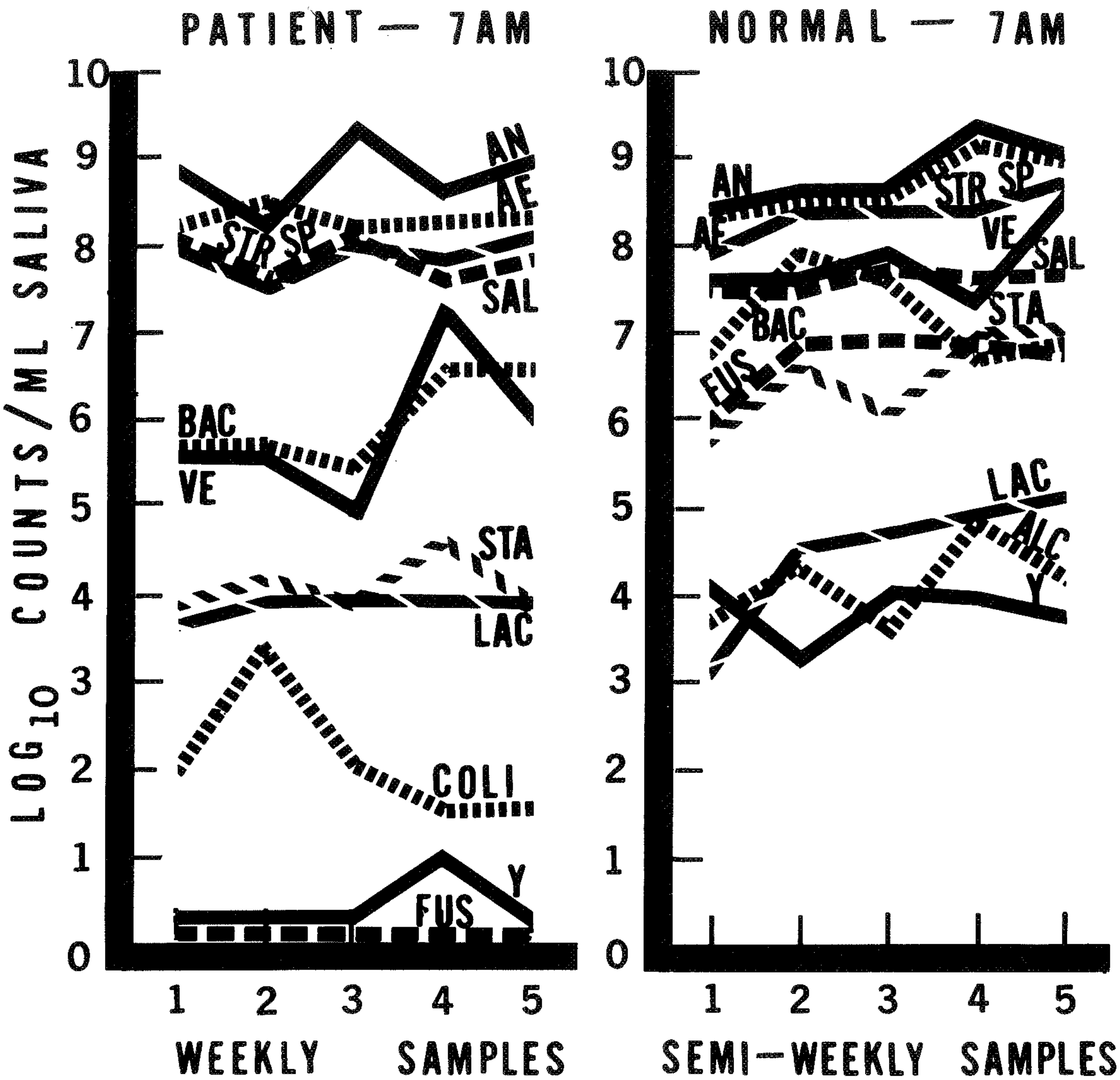


Fig. 11. Comparison of the oral microflora of a normal subject and a cancer patient confined to a conventional hospital environment.

Abbreviations: AN=anaerobes, AE-aerobes, STR SP= *Streptococcus sp.*, SAL=*Streptococcus salivarius*, BAC=bacteroides, VE=veillonella, STA=staphylococci, LAC=lactobacilli, COLI=*E. coli*, FUS=fusobacteria, ALC=*Alcaligenes*.

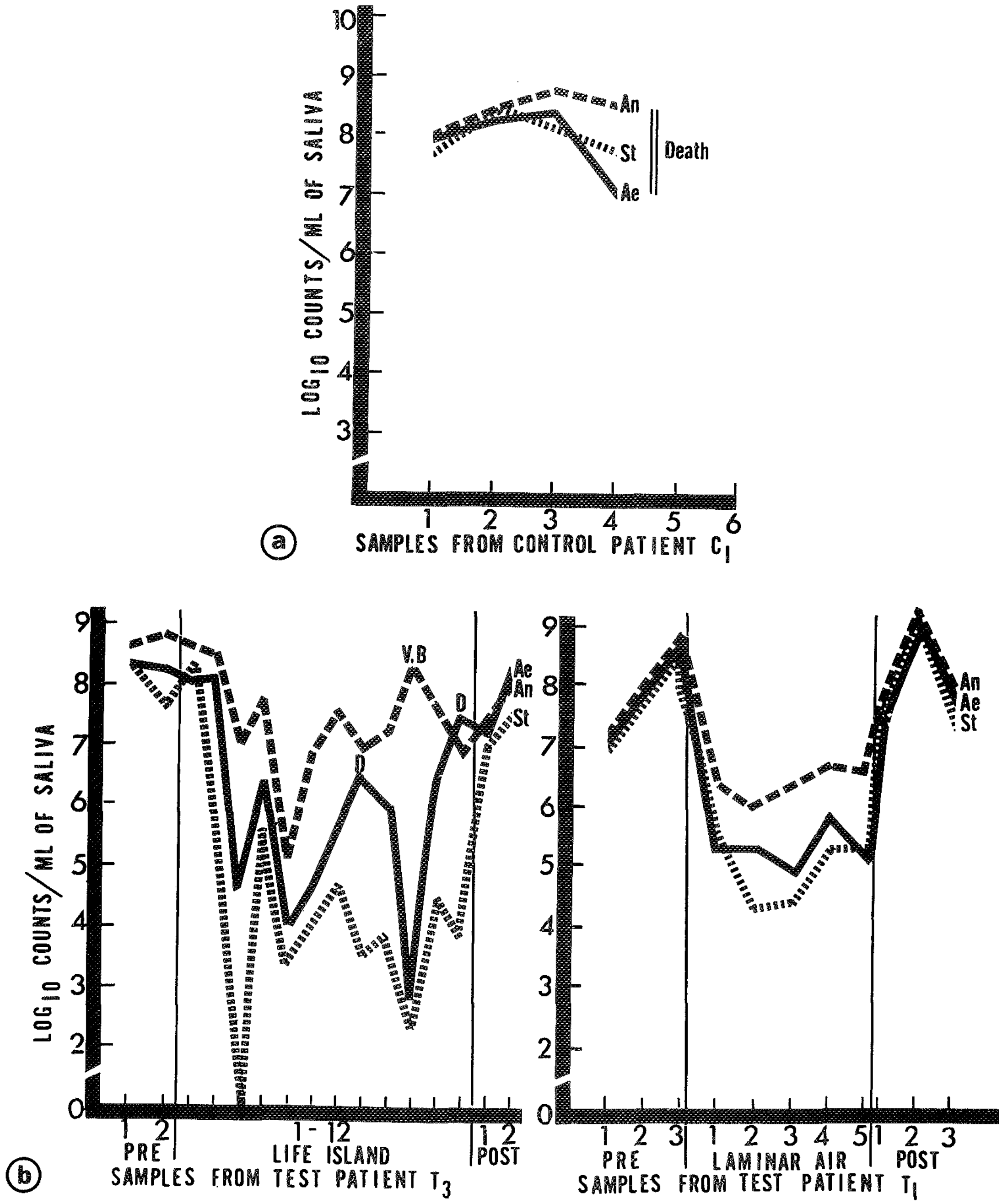


Fig. 12. A numerical comparison of predominate organisms cultured from stimulated saliva of cancer patients: (a) conventional environments, and (b) protected environments.

Abbreviations: An=anaerobes, St=streptococci, Ae-aerobes, V, B=veillonella, bacteroides, D=diphtheroids.

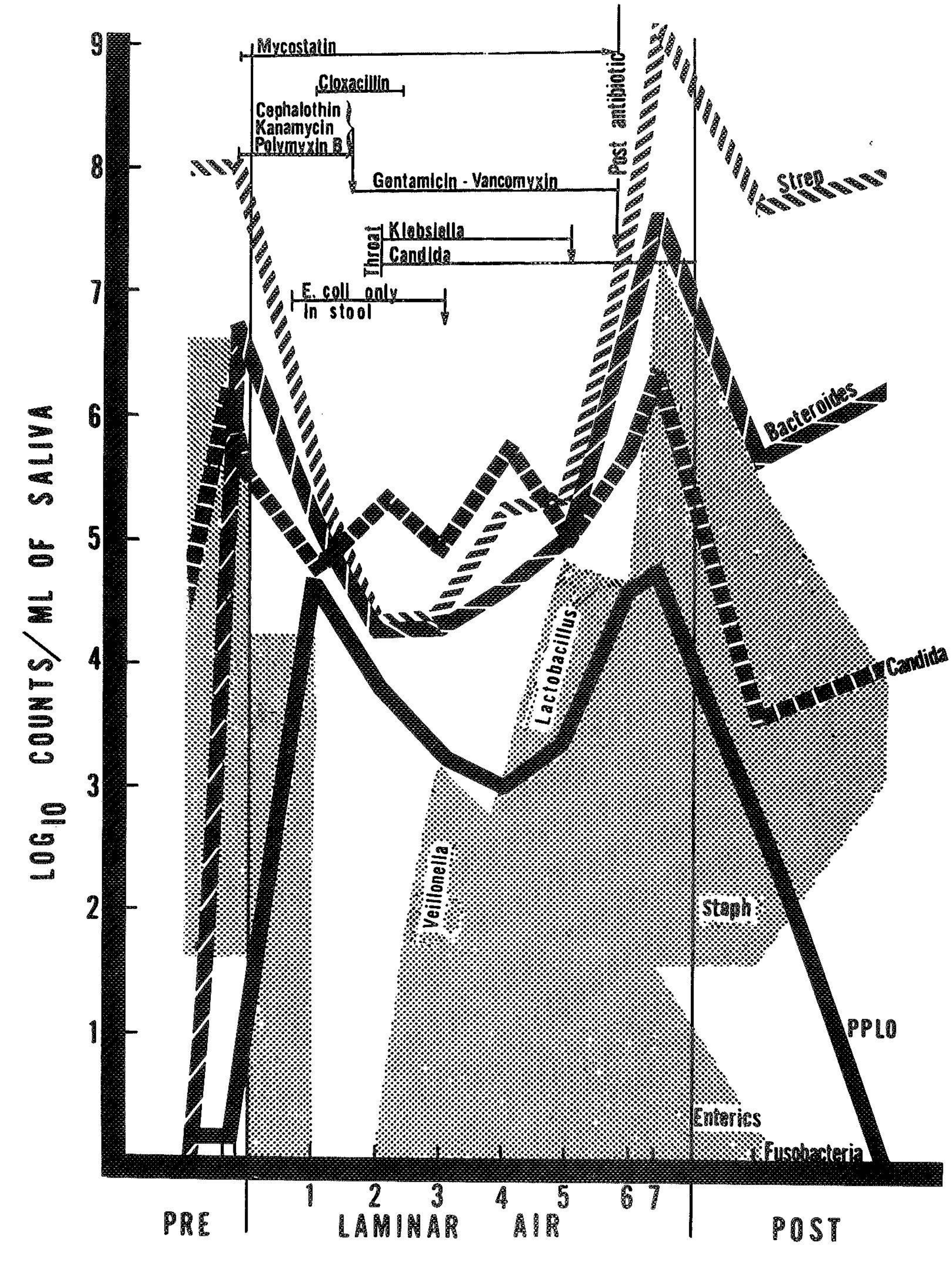


Fig. 13. Effects of intensive antibiotic prophylaxis and protected environment on the oral microflora.

Abbreviations: Strep=streptococci, Staph=staphylococci.

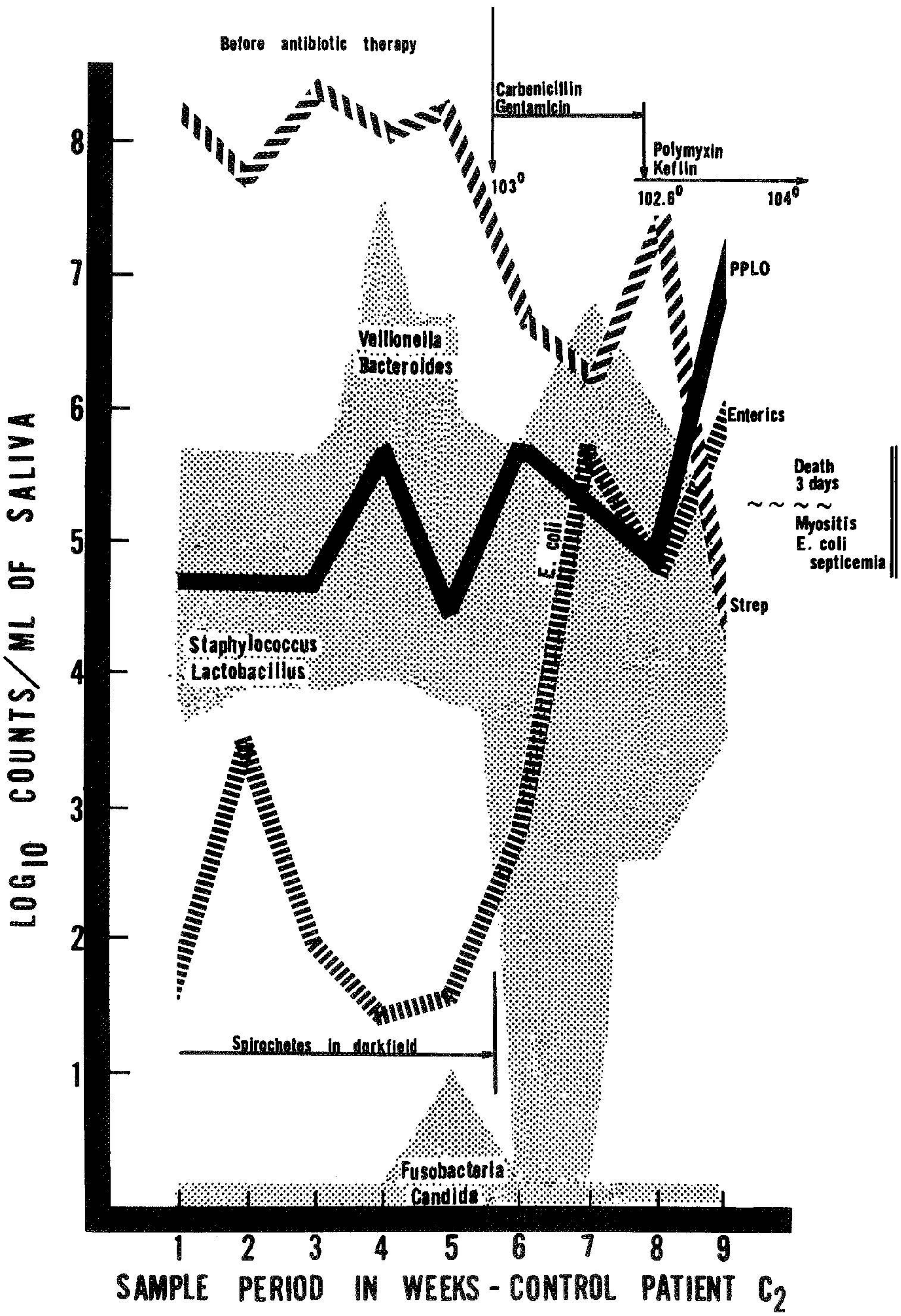


Fig. 14. Comparison of oral microbial counts to body temperature and antibiotic therapy in a patient who expired from an E. coli septicemia.

Abbreviations: Strep=streptococci.

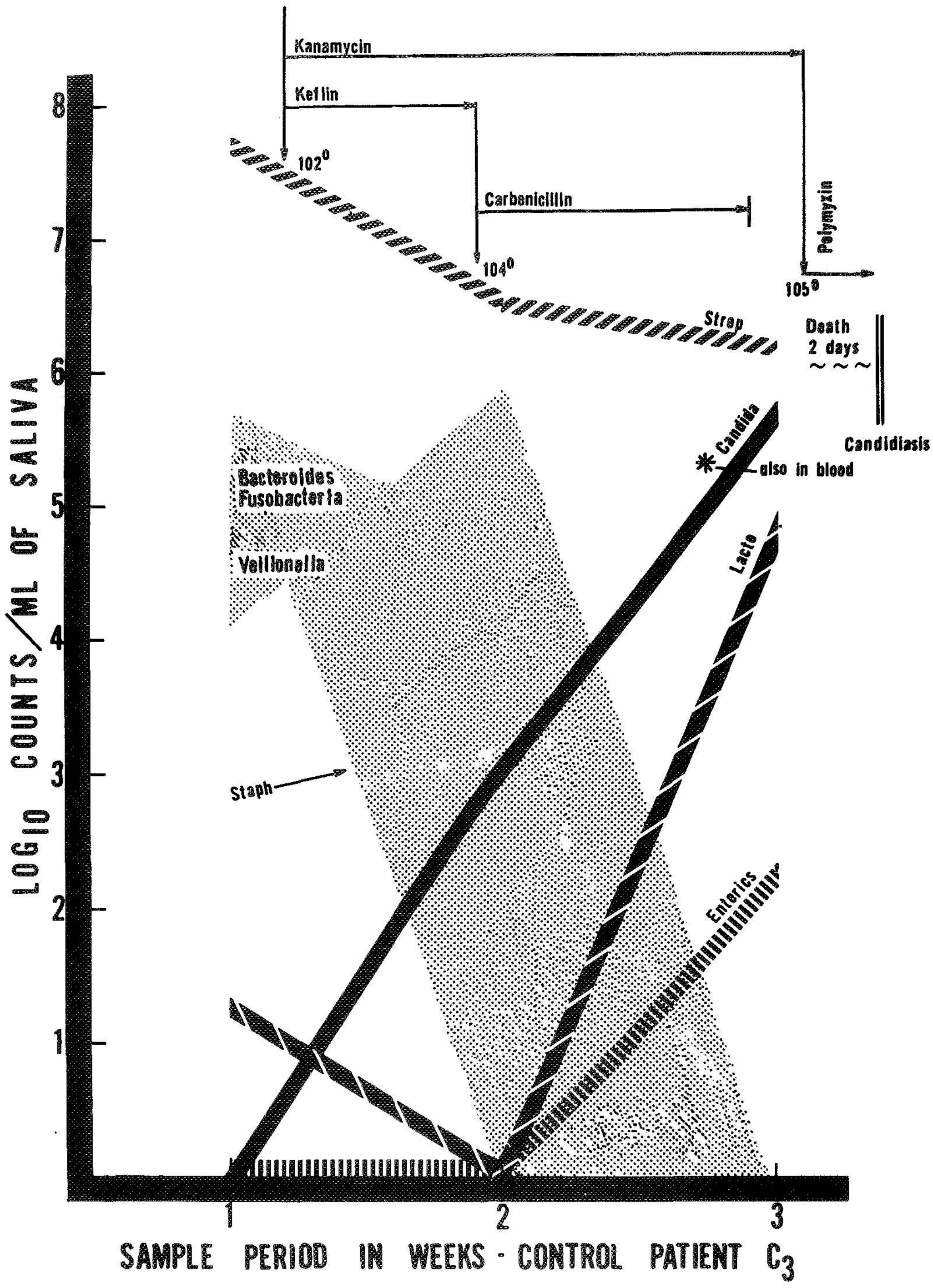


Fig. 15 Comparison of oral microbial counts to body temperature and antibiotic therapy in a patient who expired from Candidiasis.

Abbreviations: Strep=streptococci, Lacto=lactobacilli, Staph=staphylococci.

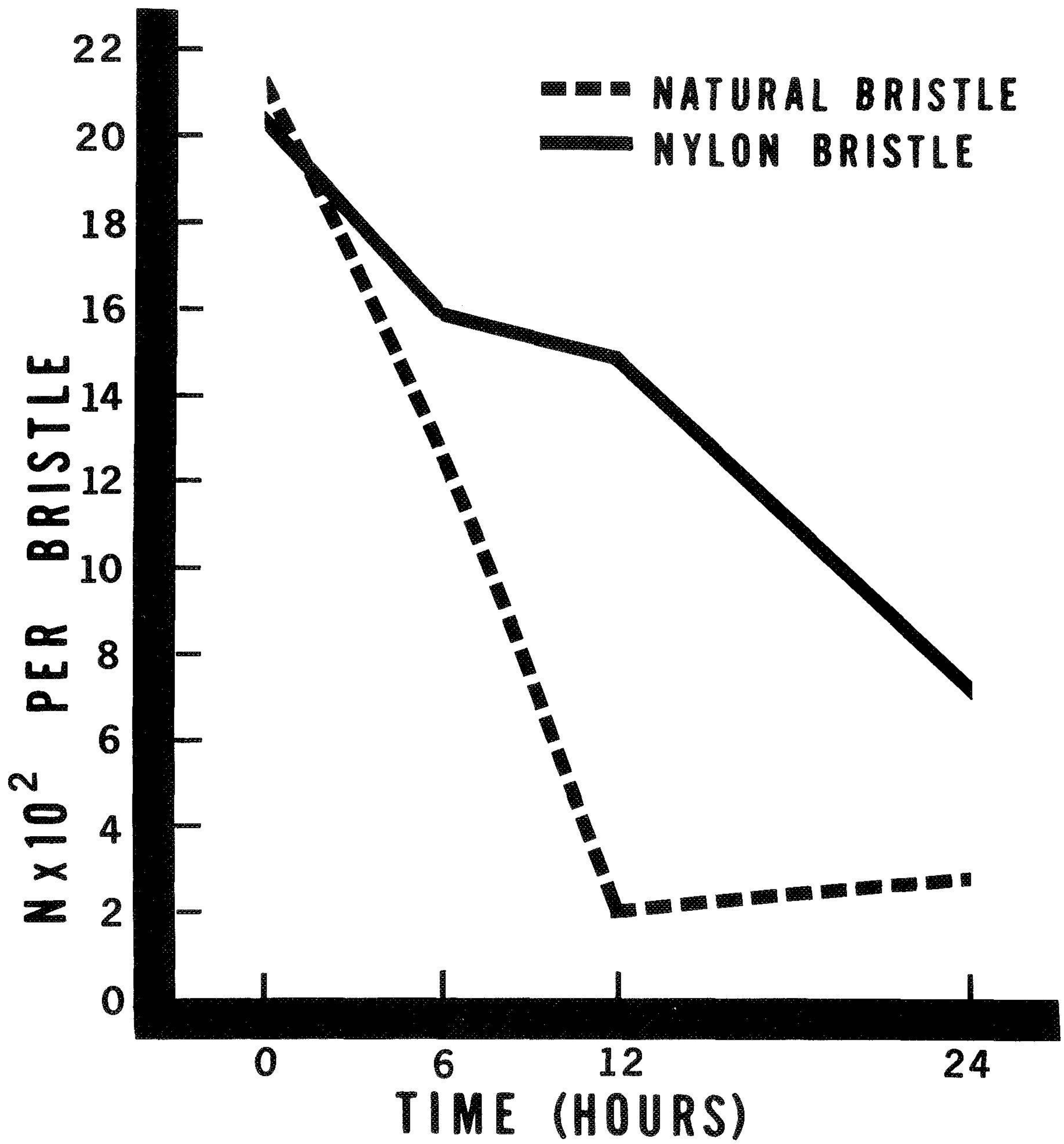


Fig. 16. Recovery of microorganisms from natural and nylon bristles of toothbrushes stored at room temperature after use.

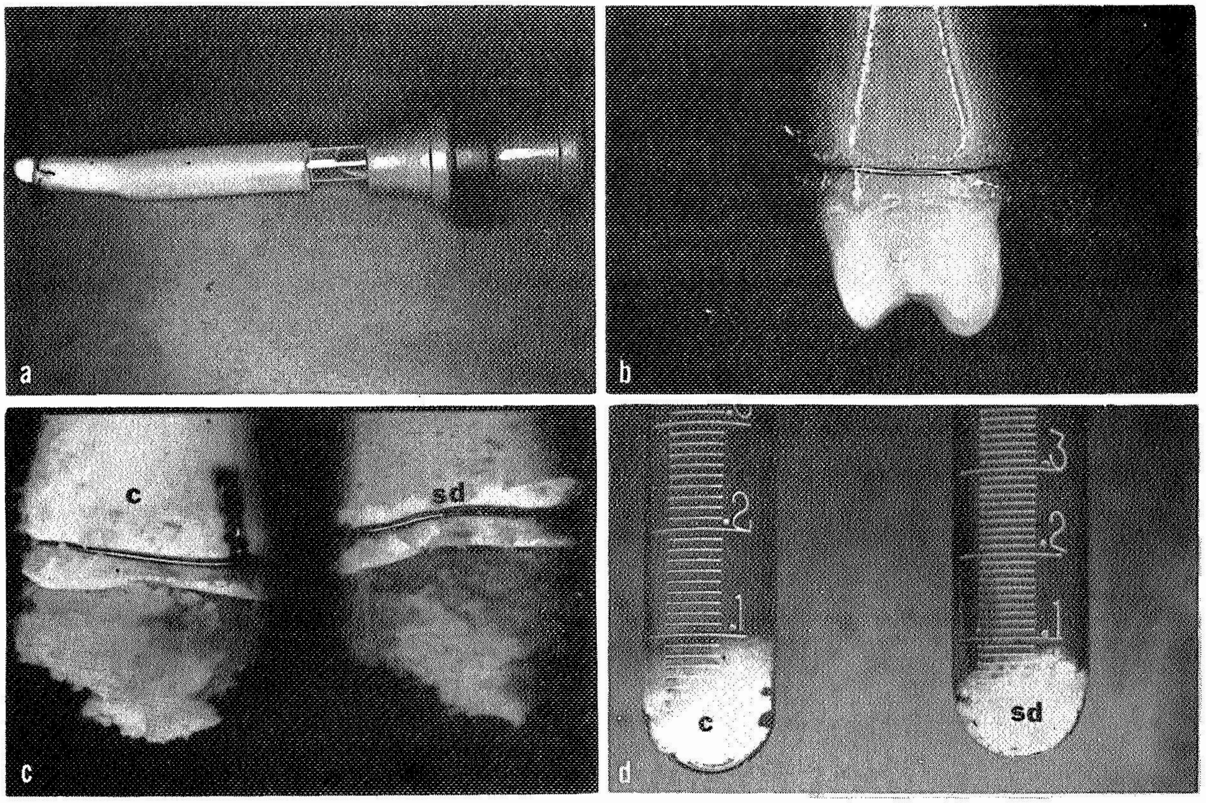


Fig. 17. Plaque formation on extracted human teeth <u>in vitro</u>: (a) mounting of an extracted tooth to a coupling of rubber and glass tubing designed to provide hydrostatic pressure in the tooth's pulp chamber, (b) magnified view of mounted tooth which represents appearance of exposed tooth surface prior to exposure to plaque forming cultures, (c) representation of plaque accumulations on a tooth exposed to control culture medium [c] and infusions from space diets [sd], (d) representative volume of plaque removed from the teeth shown in Fig. 17c.